US EPA ARCHIVE DOCUMENT

4-30-87

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005898

APR 30 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Review of submitted studies on trifluralin

To: R. Mountfort, PM-23
Registration Division

From: Marcia van Gemert, Ph.D.
Head, section III
Toxicology Branch, HED
M. van Gemert 4/28/87

Thru: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch, HED
4/29/87

Chemical: Trifluralin

Caswell #: 889

Project No: 288

Company: I. PiCi

Action Requested: Review Attached data.

The following studies were submitted by IPiCi on the technical trifluralin. A summary of the reviews follows the list of studies and the DEPs are attached. In general, most of the longer-term studies submitted have no NOELs based on liver and/or kidney toxicity seen at all doses tested.

1. Acute oral LD_{50} male rat.
2. Acute oral LD_{50} female rat.
3. Acute oral LD_{50} male and female dog.
4. Skin and mucous membrane compatibility in rabbits
5. Acute intraperitoneal LD_{50} in male rats.
6. Acute intraperitoneal LD_{50} in female rats
7. Acute dermal LD_{50} in female rats.
8. 21-day dermal study in rats.
9. Skin sensitization in Guinea pigs.
10. Ames Test
11. DNA mutagenicity test
12. Micronucleus test.
13. Dominant lethal test
14. Chromosome aberration test.
15. Gene mutation test.
16. Teratogenicity in rats.
17. Teratogenicity in rabbits
18. Multigeneration study in rats.

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19. Subchronic inhalation study in rats.
20. Subchronic study in mice
21. Subchronic study in rats.
22. Chronic toxicity study in dogs (6 months)
23. Chronic toxicity study in dogs (1 year)

Summary of the reviews which are attached.

1. Acute oral toxicity of trifluralin in male rats. Study # 706/79 Dated Nov. 8, 1979.

   LD_{50} for male rats = 1930 mg/kg
   Tox category III
   Core minimum

2. Acute oral toxicity study of trifluralin in female rats. Study # 592/79. dated Oct. 10, 1979.

   LD_{50} for female rats = 2.27 gm/kg
   Tox category III
   Core minimum

3. Acute oral toxicity of trifluralin in male and female beagle dogs. Study # 676/79, dated Nov. 5, 1979.

   LD_{50} > 10,000 mg/kg
   Tox category IV
   Core supplementary, inadequate number of animals used.

4. Skin and eye irritation study. Study # 581/79, dated Oct. 3 1979.

   Trifluralin is a slight irritant at 1 hour, and a moderate irritant after 24 hours. The peak irritation was at 7 hours in the washed eye and at 1 hour in the unwashed eye.
   Tox category III.
   Core minimum

5. Acute intraperitoneal toxicity of trifluralin in male rats. Study # 594/79, dated Oct. 10, 1979.

   LD_{50} in male rats > 5.0 gm/kg.
   Supplementary

6. Acute intraperitoneal toxicity of trifluralin in female rats. Study # 593/79, dated Oct. 10, 1979.

   LD_{50} in female rats > 5.0 gm/kg.
   Supplementary

7. Acute percutaneous toxicity of trifluralin to female rats. Study # 580/79, dated Oct. 3, 1979.

   Dermal LD_{50} in female rats > 2.0 gm/kg.
   Tox category II
   Supplementary, inadequate number of animals and only one sex used.

8. 31-day dermal toxicity study in rats. Study # 005490, dated March 3, 1982.

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NOEL = 200 mg/kg based on liver weight changes
LEL = 1000 mg/kg
Core minimum

9. Guinea pig sensitization study. Study # 84.0230, dated may 4, 1984.

No sensitization seen, but results are not definitive
Core supplementary, no positive control used.

10. Ames test. study # 74/79, dated Nov. 30, 1979.

Negative at doses up to 10,000 ug/plate, in 5 tester strains with or without S-9 activation.
Classification: acceptable.

11. Unscheduled DNA synthesis. Study # A24439, dated June 9, 1982.

Trifluralin did not induce statistically significant increases in incorporation of tritiated thymidine in the presence of hydroxyurea in cultured HELA cells with or without metabolic activation at doses up to 500 ug/ml.
Core classification: unacceptable, Not tested to cytotoxic levels, no data from preliminary tests, no repeat test.

12. mouse micronucleus test. Study # 285/81, dated may 26, 1981.

No evidence of mutagenic effects.
Core classification: acceptable.

13. Dominant Lethal test. Study # G4Mo425, Nov. 16, 1984.

No evidence of mutagenic effects.
Core classification: acceptable.

14. In vivo chromosomal aberration in Chinese Hamster. Study # M373. dated May 31, 1982.

Mutagenic effects could not be assessed because 1. the time of sacrifice did not account for mitotic delay, 2. cytotoxicity was not evident nor was clinical toxicity, 3. the experimental data requires repetition to arrive at a conclusion.

Core classification: unacceptable.

15. Gene mutation in yeast. Study # A24391, May 27, 1982.

The report mentions preliminary data from tests to arrive at the doses to be used for this study without submitting the data. In this perliminary test there was supposed to be precipitation of the test article which was proportional to the dose. These data were not supplied. The results are furnished for the percent survival of the yeast in each group. but the time of survival for these groups is not given and is required.

The data provided appear to indicate that trifluralin does not induce gene mutation in S. pombe, at the limit of solubility in standard culture medium. The study is provisionally acceptable pending receipt of the data indicated above.

16. Teratogenicity study in rats. Study # A27236, dated Oct. 18, 1983.

The NOEL and LOEL for maternal toxicity are 100 and 500 mg/kg, respectively, based on one death, clinical observations, and decreased food consumption seen at 500 mg/kg.
The NOEL for developmental toxicity could not be established due to indications of reduced skeletal maturity and increased vascular fragility in fetuses at 20 mg/kg, the lowest dose level tested. Additional developmental effects were total litter resorption shortly after implantation at 100 mg/kg and reduced fetal weights and lengths and increased resorption rates at 500 mg/kg.
Since a NOEL for developmental toxicity was not determined, this study was classified core supplementary.

17. Teratogenicity study in rabbits. Study # A29709, dated Aug. 9, 1984.

The absence of toxic effects in this study, even at the highest dose level tested (60 mg/kg), precluded assessment of the LOEL for maternal and developmental toxicity of trifluralin. The study was classified core supplementary due to the lack of toxic effects. In the event that further work is conducted, it is recommended that:
1. higher dose levels be used to achieve some maternal toxicity as suggested by the USEPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation, Nov. 1982.
2. Data from chemical analyses of dosing suspensions be reported.
3. The animals be randomized in a manner that ensures comparable initial body weights among all groups.

18. 2-generation reproduction study in rats. Study #C08875, dated Oct. 17, 1984.

The NOEL for parental toxicity of trifluralin in rats could not be determined due to increased relative kidney weights at all dose levels tested (i.e., 200, 650, and 2000 ppm), renal lesions of the proximal tubules and increased relative liver weights at 650 and 2000 ppm, one death due to acute renal failure at 650 ppm, and reduced body weights at 2000 ppm. The NEOL for this study was 200 ppm.
The NOEL and LOEL for reproductive and developmental toxicity were 200 and 650 ppm, respectively, based on reduced weanling body weights at 650 and 2000 ppm and reduced litter sizes at 2000 ppm. The study is classified core minimum.

19. Subchronic Inhalation study in rats. Study #5488, dated Feb. 12, 1982.

Doses tested in nose-only inhalation study with exposure 6 hours/day, 5 days/week were 100, 301 and 1006 mg/CBM. Effects were detected in high dose group showing signs of toxicity 6 hours after the

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initial exposure. There was an increase in group 4 methemoglobin levels, however, the data were not presented to confirm or deny this statement. Total bilirubins were increased in the mid and high dose groups. The study stated that direct bilirubins were also elevated, but did not give the data to confirm this statement. [illegible] high dose male and female absolute and relative liver weights were increased, and centrilobular hypertrophy of the liver [illegible]n in high dose females and at all doses for males.
NOEL 100 mg/CBM (LDT) based on centrilobular hypertrophy seen in males at all doses tested.

20. Subchronic toxicity study in mice. Study #008842, dated June 15, 1983.

There is no NOEL for liver-to-body weight ratios in female mice. Albumin levels were decreased at all doses in mice, however, the significance of this is not known. Only limited histopathology was performed in this study. It was basically a rangefinding study for the chronic study.
NOEL < 400 ppm
Core supplementary.

21. Subchronic feeding study in rats. Study #0620, dated Nov. 18, 1980.

There are treatment-related trends and effects in several parameters including female liver/body weight increases and pituitary to body weight decreases with no NOEL.
Core minimum.

22. 6-month subchronic toxicity study in dogs. Study #633, dated Oct. 30, 1981.

Under the conditions of the study, trifluralin was toxic to male and female dogs when fed for 6 months at levels of 400, 1000, or 2500 ppm in the diet. The following compound-related effects were observed at all dose levels: enlarged livers, discolored kidneys, corneal vascularization, hemolytic anemia and increased serum alkaline phosphatase activity. An exceeded maximum tolerated dose (MTD) and aversion to food caused starvation in two high-dose animals (one male, one female) and resulted in their moribund sacrifice after 86 and 39 days of dosing, respectively. Reductions in body weight gain and food consumption were observed in the high-dose animals. There were no distinct or consistent histologic alterations that could be related to the increased liver weights and discoloration of kidneys. In some high-dose males, testes were smaller than normal.
The NOEL for systemic toxicity was not achieved; the LOEL is 400 ppm, the lowest dose tested.
Core supplementary.

23. Chronic (1-year) feeding study in Dogs. Study #A29701, dated Nov. 9, 1984.

When trifluralin was fed to dogs for 1 year at dietary levels of 30, 150 or 750 ppm, there was a decreased weight gain in males and females receiving 750 ppm. There were some significant

decreases in red blood cell parameters in high dose males and females and an increase in methemoglobin. Total serum lipids, triglycerides, and cholesterol were increased in high-dose males and females when compared to controls. There were increases in liver weight in males receiving 150 and 750 ppm and females receiving 750 ppm trifluralin and increases in mean spleen weight in females receiving 750 ppm. There were no histologic findings that correlated with the organ weight changes. Based on the increases in liver weights, the LOEL is 150 ppm and the NOEL is 30 ppm.
Core Guideline.

DATA EVALUATION REPORT

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Citation:

Acute Oral Toxicity of Trifluralin (HOE 38474 OH AT 204) to the Male Rat, Dr. Hollander, Dr. Weigand, Pharma Forschung Toxicologie, Hoechst, Frankfurt, Germany, November 8, 1979, Report No. 706/79.

Materials:

Test Material: HOE 38474 OH AT204, a red-orange crystalline substance 25 percent solution in sesame oil. The purity of the substance was not given.

Animals: Male Wistar rats (strain HOE WISKf SPF71) from Hoescht breeding stock, weighing 174 - 202 g.

Methods:

The animals were allowed Altromin 1324 diet and tap water. The animals were fasted for 16 hr. prior to dosing and for an additional two hours post dosing. Ten rats (5 of each sex) per dosage group (1250, 1600, 2000, 3150, and 5000 mg/kg given in a single dose by gavage) were observed for 14 days.

The signs of toxicity, mortality rate, and time of death were recorded. The animals were weighed weekly. The animals that died on test were discarded after macroscopic examination.

The LD_{50} was determined by probit analysis.

Results:

As tabulated in the report the results are as follows:

Dose mg/kg	%trifluralin solution	Mortalities/No. of Animals
1250	25	0/10
1600	25	1/10
2000	25	6/10
3150	25	10/10
5000	25	10/10

Symptoms:

The mortalities occurred within 1 to 3 days of treatment with the following symptoms: motor unrest, squatting position, bristled hair, loss of equilibrium, trembling, Dalrymple's sign, mydriasis, exophthalmos, and increased lacrimation. The survivors at 48 hours had no clinical symptoms, at 48 hours after treatment. For the remainder of the 14 day survival period the behavior and body weight changes were found to be as expected for untreated animals.

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Macroscopic pathology of the mortalities revealed some translucence of the liver and spleen, discernible markings of the lobules of the liver, orange to yellow discoloration of fat in the abdomen, gastrointestinal tract filled with an orange substance, and blood red excretions from mouth and nose.

Conclusion:

The oral median LD_{50} for the male rat was 1930 mg/kg (p = 0.05, 1750 - 2380 mg/kg).

Toxicity Category: III

Classification: Core Minimum

Reviewed by: Stephanie P. April, Ph.D. Stephanie P April

Date: 6/16/86 as revised to 11/26

Secondary Reviewer: Marcia Van Gemert, Ph.D. M. VanGemert 7.1.86

Date:

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DATA EVALUATION REPORT

Citation:

Acute Oral Toxicity of Trifluralin (HOE 38472 OH AT204) to the Female Rat, Dr. Hollander, Dr. Weigand, October 10, 1979, Report No. 592/79.

Materials:

Test Material: HOE 38474 OH AT204, a red-orange crystalline substance as a 25 percent solution in sesame oil. The percent purity was not given.

Animals: Female Wistar rats (strain HOE WISKf SPE71) from Hoechst breeding stock weighing 170 to 190 g were used in groups of 10 rats per dosage.

Methods:

The animals were fasted for 16 hours prior to and 2 hours posttreatment. Food and water were available during the 14 days following treatment.

The animals were observed for symptoms of intoxication, mortality rate, and time for death. The animals were weighed weekly. The animals were examined macroscopically on death or sacrifice.

The LD_{50} was determined by probit analysis (Linder/Weber method) while confidence limits were calculated according to Fieller.

Results:

A tabulation of the male mortalities is given as follows:

Dose mg/kg (25% trifluralin solution)	Mortalities/No. of Animals
1250	0/10
1600	1/10
2000	4/10
3150	7/10
5000	10/10

Deaths occurred within 3 days of treatment with these symptoms: motor unrest, squatting, bristled hair, disequilibrium, abdominal position, trembling, Dalrymple's sign, exophthalmos, mydriasis, and lacrimation. The survivors were symptom-free within 48 hours of treatment, and remained so for 14 days.

There was no difference from control in posttreatment weight gain nor were there any behavior abnormalities in the treated animals.

The gross pathological exam findings of animals dead on test were indicated to be: naked sites on the body, connective tissue and fatty tissue in the abdominal cavity, yellow discoloration of intestinal walls, slight marking of hepatic lobules, stomach contained excessive amounts of yellow feed mash, and autolysis; the surviving rats had slightto marked yellow discoloration of the fatty tissue in the abdominal cavity.

Conclusion:

The oral LD_{50} in the female rat was calculated as 2.27 g/kg. Confidence range for p = 0.05 1.760 to 2.870 g/kg is confidence range. The regulatory requirement for use of both sexes is fulfilled where the two studies with male alone and female alone are considered.

Category: III

Toxicity Classification: Core Minimum.

Review by: Stephanie P. April, Ph.D.
SPA

Date: 6/16/86 delivered to M.U.G

Secondary Reviewer: M. MacQueen 7.8.86

Date: keyed by me 7.3.86

DATA EVALUATION REPORT

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Citation:

Hollander and Weigand, Acute Oral Toxicity of Trifluralin (HOE 38474 OH AT204) in the Male and Female Beagle Dog, Hoechst, Report No. 676/79, November 5, 1979.

Materials:

Test Material: Trifluralin (HOE 38474 OH AT204) as a red-orange, crystalline substance, dissolved in sesame oil.

Animals: Pure bred male and female beagle dogs (Hoechst breeding) weighing from 11.9 to 17.7 kg were used.

Methods:

The dogs were fasted for 16 hours prior to treatment and 5 to 6 hours after treatment. During the remaining posttreatment observation period of 14 days the dogs who were singly housed received food and water ad libitum. The dogs were inspected for behavior and the general state of health. The weights were recorded three times a week.

Animals (one male and one female per group) received a single dose of 2000, 3000, or 10,000 mg/kg by gavage.

Results:

The reported results were:

Dose mg/kg	Mortalities/No. of Animals
2000	0/2
3000	0/2
10,000	0/2

At 2000 and 3000 mg/kg behavior and body weights were normal but red-orange to light yellow colored feces and diarrhea were observed.

At 10,000 mg/kg there was a decrease in body weight by 0.5 kg of the male dog 48 hours after treatment in addition to the symthoms found at 2000 and 3000 mg/kg. There were no further abnormalities found in the follow up period.

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Conclusion:

The oral LD_{50} was greater than 10,000 mg/kg in the dog in this study.

Toxicity Category: IV

Classification: Supplementary, inadequate numbers of animals used.

Reviewed by: Stephanie P. April, Ph.D.

Date: SPA
6/10/86 delivered to M.V.G.

Secondary Reviewer: Marcia Van Gemert, Ph.D.

Date: M. Van Gemert 7.8.86
Rec'd by me 7.3.86

DATA EVALUATION REPORT

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Citation:

Irritance to the Rabbit Skin and Eye Mucosa, Dr. Hollander and Dr. Weigand, October 3, 1979, Report No. 581/79.

Materials:

Test Material: Trifluralin (HOE 38474 CH AT204) as red-orange crystalline substance was used.

Animals: Albino-Himalayan rabbits of strain HOE:HIMK (SPF Wiga) from Hoechst breeding stock weighing 1.5 to 2.0 kg were used.

Method, for Skin Irritation:

Irritation to the Skin:

An area 6 x 3 cm was shaved on the flank skin of six rabbits. One-half of the area was abraded. Five-hundred mg of test material in 0.15 mL of PEG 400 was applied to cellulose patches which were taped to the test area and covered with PVC foil. The trunk of the animal was bandaged with Elastoplast for 24 hours.

The results were evaluated immediately upon bandage removal and at 48 and 72 hours after treatment.

Results:

The primary irritation index given in the report was 1.4 based upon the following grading system as given in the report.

1. Erythema and eschar formation	Value
No erythema	0
Very slight, barely perceptible erythema	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight Eschar formation (injuries in depth)	4

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2. Edema formation

No Edema	0
Very slight, barely perceptible edema	1
Slight edema, edges of area well-defined by definite raising	2
Moderate edema, raised appr. 1 mm	3
Severe edema, raised more than 1 mm and extending beyond the area of exposure	4

The values for erythema and eschar formation at 24 hour and 72 hours for intact skin are added to the values on abraded skin at 24 and 72 hours (four values). Similarly, the values for oedema formation at 24 hours and 72 hours for intact and abraded skin (four values) are added. The total of these eight values is divided by four to give the primary irritation score.

0.0 - 0.5	nonirritant
0.6 - 3.0	slight irritant
3.1 - 5.0	moderate irritant
5.1 - 8.0	severe irritant

The individual results for each animal that are given in the report are as follows:

Animal No.	930	931	932	964	965	947
24 Hr.						
Abraded Erythema	1	1	1	2	2	1
Abraded Edema	1	1	1	2	1	1
Intact Erythema	1	1	1	1	1	1
Intact Edema	1	0	0	0	1	1
48 Hr.						
Abraded Erythema	1	1	1	2	2	1
Abraded Edema	0	0	0	1	0	1
Intact Erythema	1	1	1	0	1	1
Intact Edema	0	0	0	0	0	1
72 Hr.						
Abraded Erythema	1	1	1	1	1	1
Abraded Edema	0	0	0	0	0	0
Intact Erythema	1	1	1	0	0	1
Intact Edema	0	0	0	0	0	0
Totals	6	+ 5	+ 5	+ 6	+ 6	+ 6 = 34

Total

No. of animals x 4 = $\frac{34}{6 \times 4}$ = 1.4 primary

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Conclusion:

Trifluralin is a slight irritant to the skin.

Toxicity Category: IV

Classification: Core-Minimum

Methods for Irritance to Eye Mucosa

Nine rabbits received single doses of 100 mg Trifluralin (100 mg in PE6400) in the conjunctival sac of the left eye. The right eyes of the animals provided an individual self control. The left eyes of three animals were flushed with physiological saline after 1 minute of treatment. The left eyes of the remaining six animals were not washed.

The eyes were evaluated at 1, 7, 24, 48, and 72 hours after Trifluralin treatment. Fluoresecein-sodium (0.01%) was instilled in the eyes prior to the 48- and 72- hour evaluations. The classification system used in evaluating the eyes is that reported in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics," FDA, Austin, Texas, pg. 51, 1975 Appendix 2 given below:

Appendix 2 Scale for Scoring Ocular Lesions	Score
(1) Cornea	
(A) Opacity-degree of density (area most dense taken for reading)	
No Opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque, iris invisible	4
(B) Area of cornea involved	
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three-quarters	3
Greater than three quarters, up to whole area	4
A X B X 5	Total maximum = 80

Appendix 2 Scale for Scoring Ocular Lesions (Continued) — Score

(2) Iris

(A) Value

	Score
Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, hemorrhage, gross destruction (any or all of these)	2

A X 5 — Total maximum = 10

(3) Conjunctivae

(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

	Score
Normal	0
Definitely inflamed above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse beefy red	3

(B) Swelling

	Score
No swelling	0
Any swelling above normal (including nictitating membrane)	1
Obvious swelling with partial closing of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

(C) Discharge

	Score
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) X 2 — Total maximum = 20

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Results:

From the reported individual scores for washed eyes the highest irritation index was found to be 17 out of 110 which corresponds to a slight irritation of the mucosa while the highest irritation index found in the unwashed eyes was 26 after 1 hour.

The eye irritation index is the sum of the readings for the cornea, iris, and conjunctiva at any set time. The following scores are classified as given below:

0 - 10	nonirritant
11 - 25	slight irritatant
25 - 56	moderate irritant
57 - 110	severe irritant

Conclusion:

Trifluralin is a slight irritant at 1 hour, and a moderate irritant after 24 hours. The peak irritation was at 7 hours in the washed eye and at 1 hour in the unwashed eye.

Toxicity Category: III.

Classification: Core Minimum.

Reviewed by: Stephanie P. April, Ph.D. SPA

Date: 6/13/86

Secondary Reviewer: M. Mansfield

Date: 7.1.86

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DATA EVALUATION REPORT

Citation:

Acute Intraperitoneal Toxicity of Trifluralin (HOE 38474 OH AT204) in the male rat, Dr. Hollander, Dr. Weigand, Hoechst, Frankfurt, Germany, October 10, 1979, Report No. 594/79.

Materials:

Test Substance: A 25 percent solution of Trifluralin (HOE 38474 OH AT 204), a red-orange colored crystalline substance in sesame oil.

Animals: Female Wistar rats (strain: HOE WISKf (SPF71) from Hoechst breeding farm) weighing 190 to 208 g were used.

Methods:

A single intraperitoneal dose of 5 gm/kg was given to 10 male rats. For the 14 days following treatment symptoms of intoxication and mortality were recorded. The animals were weighed weekly posttreatment. The survivors were sacrificed after the 14-day observation period and examined macroscopically.

Results:

There were no deaths and behavior and body weight gains were normal. There were no clinical symptoms 24 hours after treatment. The observed symptoms were motor unrest, squatting, bristled hair, abdominal position, and passiveness.

Observations from the macroscopic postmortem examination were intense yellow coloration of connective and fatty tissue in the abdominal cavity.

Conclusion:

The intraperitoneal LD_{50} in the male rat was found to be greater than 5.0 gm/kg.

Classification: Supplementary, there is no Guideline requirement for an intraperitoneal LD_{50}.

Reviewed by: Stephanie P. April, Ph.D. Stephanie April

Date: ~~5/5/86~~ 6/5/86 2Ph secondary date validated

Secondary Reviewer: Marcia Van Gemert, Ph D. Marcia Van Gemert 6.9.86 Rec'd by me 6.5.86

Date:

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DATA EVALUATION REPORT

Citation:

Acute Intraperitoneal Toxicity of Trifluralin (HOE 38474 OH AT204) in the female rat, Dr. Hollander, Dr. Weigand, Hoechst, Frankfurt, Germany, October 10, 1979, Report No. 593/79.

Materials:

Test Substance: A 25 percent solution of Trifluralin (HOE 38474 OH AT 204), a red-orange colored crystalline substance in sesame oil.

Animals: Female Wistar rats (strain: HOE WISKf (SPF71) from Hoechst breeding farm weighing 200 to 218 g were used.

Methods:

A single intraperitoneal dose of 5 gm/kg was given to 10 female rats. For the 14 days following treatment symptoms of intoxication and mortality were recorded. The animals were weighed weekly posttreatment. The survivors were sacrificed after the 14-day observation period and examined macroscopically.

Results:

There were no deaths and behavior and body weight gains were normal. There were no clinical symptoms 24 hours after treatment. The observed symptoms were motor unrest, squatting, bristled hair, abdominal position, and passiveness.

Observations from the macroscopic postmortem examination were intense yellow coloration of connective and fatty tissue in the abdominal cavity.

Conclusion:

The intraperitoneal LD_{50} in the female rat was found to be greater than 5.0 gm/kg.

Classification: Supplementary, there is no Guideline requirement for an intraperitoneal LD_{50}.

Reviewed by: Stephanie P. April, Ph.D.

Date: Stephanie April ~~6/5/86~~ 6/5/86 SPA reviewed data unsatisfactorily

Secondary Reviewer: Marcia Van Gemert, PhD Marcia Van Gemert 6.5.86 rec'd by me 6.5.86

Date:

DATA EVALUATION REPORT

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Citation:

Acute Percutaneous Toxicity of Trifluralin - HOE 38474 OH AT204 to the Female Rat, Dr. Hollander and Dr. Weigand, Hoechst Laboratories, Frankfurt, Germany, October 3, 1979, Report No. 580/79.

Materials:

Test Substance: Trifluralin-HOE 38474 OH AT204 was used as a red-orange substance; however, the physical form for application was not indicated.

Animals: Six female Wistar rats (strain: HOE WISKf (SPF71) weighing 170-186 g from Hoechst breeding stock were used.

Methods:

The test substance (2.0 gm/kg) was applied to the shaven dorsal skin of 6 individually housed females. After application the treatment area was covered with aluminum foil covered with an elastic plaster bandage for 24 hours. After this time the dressing was removed and the treated site was washed.

The symptoms of intoxication were recorded. The animals were observed for 14 days and weighed weekly.

Upon death, the animals were autopsied; carcasses were macroscopically examined.

Results:

There were no deaths, symptoms of intoxication, abnormal behavior or reduction of body weight gain associated with dermal trifluralin application in this experiment. There was however, yellow skin coloration from trifluralin in 24 hours. Autopsy revealed no abnormal findings.

Conclusion:

The dermal LD_{50} female ~~rabbits~~ RATS is >2.0 gm/kg in this experiment.

Toxicity Category: Category II

Classification: Supplementary, did not use an adequate number of animals and both sexes.

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Reviewed by: Stephanie P. April, Ph.D.

Date: 6/26/86 SPA

Secondary Reviewer: Marcia Van Gemert, Ph.D. M Van Gemert 7.1.86

Date:

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DATA EVALUATION REPORT

Citation:

Thirty-one-day Dermal Toxicity Study with HOE 38474 OH at 210 active ingredient <Technical> in Rats, Dr. K. H. Laist, Research Consulting Company, Ltd., Itingen, Switzerland, Project No. 005490; March 3, 1982.

Materials:

Test Substance: HOE 38474 OH AT210 active ingredient (Technical) was used.

Animals: Male and female Wistar KFM-HAN outbred SPF Quality rats were used.

Methods:

Ten male and ten female rats per group were tested for 31-day dermal toxicity of trifluralin with 13-day recovery period. Doses of 0, 40, 200 and 1000 mg/kg trifluralin was applied daily as a 50 percent suspension in 2 percent solution of carboxymethyl cellulose and sodium salt purum to 10 percent of the body's dermal surface (clipped dorsal surface) for 6 hours. The animals were weighed initially and weekly thereafter during the 31-day treatment period. There was a total of 23 applications.

The animals were observed for mortality, signs of local and systemic toxicity and skin irritation. Food and water consumption were recorded as well as body weight gain and food conversion data.

There was an ophthalmic examination before treatment and at the end of the trial period. Blood samples were taken to assess the hematological and blood chemistry values after 31 days of treatment and after the 13-day recovery period.

Organ weights were recorded for all groups at autopsy. Histopathological examination was performed on 30 tissues from all animals in the control and dose groups as well as animals that died on test in the other groups.

Statistical analysis was performed on the data and recorded.

Results:

There were no deaths during the 44 days of the test periods in this study.

There were no observable signs of local and/or systemic toxicity nor any skin irritation on the areas of application.

In this study there was no treatment related statistically significant differences from control in food consumption, water consumption, body weight gain or food conversion in gm consumed/body weight/day.

The haematology and biochemistry data obtained from this study were unremarkable.

Organ weights of brain, heart, kidneys, gonads, and adrenals did not fluctuate significantly among any of the groups.

After 31 days the liver in the high dose males increased in weight. The absolute liver weight increase was not significant; however, the liver to body weight ratio increased significantly ($p < 0.01$) as did the liver to brain weight ratio ($p < 0.05$). After recovery the liver to body weight ratio increase was only borderline significant ($p < 0.01$, > 0.05).

The macroscopic pathological examination did not reveal any treatment related reports.

Histopathological examination indicated an increase in damage to the site of dermal application of trifluralin as exemplified by hyperkeratosis, acanthosis, sebaceous gland hyperplasia, dermal ulcerations and inflamed epidermal junctions.

Glycogen storage occurred in livers in all groups in a nondose-related manner and the high dose males had a nonsignificant minimal increase.

Hyaline droplets in proximal tubular epithelium of the kidney were found in all treated male groups and a few female treated groups in a nonsignificant, not dose related way. There were more droplets in the high dose males than in the control group.

No other significant lesions were found.

Conclusion:

In this study, the 31-day dermal application of trifluralin to rats, the NOEL was ~~> 1000 mg/kg/day~~. 200 mg/kg Based on liver change.

Classification: ~~Supplementary, there is no core guideline for this type of study~~. Minimum

005898

Reviewed by: Stephanie P. April, Ph.D. Stephanie P April.

Date: ~~5/6/86~~ 6/5/86 received date read verbatim 6/10/86

Secondary Reviewer: Marcia Van Gemert, Ph.D. M. Van Gemert 6.9.86

Date: rec'd by me 6.5.86

005898

DATA EVALUATION REPORT

Citation:

Trifluralin test for sensitizing properties in Pirbright-White guinea pigs according to the method of Buehler, Dr. Rupprich and Dr. Weigand, Hoechst AG, Report No. 84.0230, May 4, 1984.

Materials:

Test Substance: Trifluralin, HOE 038474 OH ZD99 0002 as orange crystals, 98.4 percent pure, Batch No. 11710 was used.

Animals: Female Pirbright-White guinea pigs strain HOE: DHPK (SPFLac) approximately 255.1 gm from Hoechst AG, Kastengrund, SPF breeding colony were used in this study.

Methods:

The animals were divided into 10 animals in the control group and 20 animals in the treatment group for use in the sensitizing test according to Buehler. The test substance was dissolved in petrolatum (Vaseline). The test concentration was determined by primary preliminary tests to find a nonirritant concentration. The method of Magnusson and Kligman determined that such a concentration of trifluralin in petrolatum was 10 percent (Industrial Toxicology Report No. 84.0009).

Initially body weights of the animals were recorded. The front part of the left flank was shaven in preparation for the dermal application of the test material at a sensitizing dose which was made fresh daily as 0.5 ml of 50 percent solution in petrolatum on a 2 x 2 cm cellulose patch. The patch was covered with an occlusive polyethlene film and a bandage. The control animals received vehicle alone. After 6 hours exposure the bandage was removed and the skin washed with polyethlene glycol 400 and water 1:1 mixture. Clinical signs and irritating effects were recorded. This was repeated 9 times 3 times a week for 3 weeks. The animals received no further treatment from day 20 to day 35.

On day 36 the first challenge treatment (i.e., the primary nonirritant concentration) for both the control and treatment group was applied to the previously untreated shaved right flank in the same way as before. The flank was examined then shaved again and reexamined the next day. Simultaneously the rear right flank was challenged with 0.5 ml of a 10 percent solution of the test material. On day 44 the second challenge of 5 percent solution of HOE 038474 OH 2D99 009 in petrolatum was applied to the rear part of the untreated right flank. The terminal body weight was recorded.

Criteria for an allergic reaction were mainly erythema and edema.

The relative number of treated and control animals reacting to the signs of irritation is used as criteria for evaluation of sensitizing properties of a test substance - the percentage difference between the two groups. It is stated that a 15 percent reaction of the treated group indicates a definitely positive reaction.

Results:

1. Sensitization Period

The body weight gains of both groups of animals were normal and no clinical signs of intoxication were observed. Irritation was seen after 2 or 3 applications as slight to well-defined reddening. After 5 to 6 treatments the skin was dry, friable and in some papyraceous. One animal at this point had desquamation at the area of application. The signs of irritation increased with repetitive treatment.

2. First Challenge

When challenged with the sensitizing dose (the primary nonirritant concentration) 2/10 of the control group had barely perceptible erythema 24 hours after challenge and reversible 48 hours after application.

After 24 hours the treated animals showed 4/20 pronounced erythema and another 7/20 barely perceptible erythema. There was very slight edema in 2 animals. One had dry, friable skin and 2 others reddened in the entire flank. These effects were almost entirely reversible in 48 hours.

3. Second Challenge

Using one-half the sensitizing dose in the control group evoked no signs of irritation. In the treated group only 1 of the 20 animals showed pronounced erythema at 24 hours which persisted with reduced intensity to 48 hours.

Discussion:

The 10 percent dose which was found to be definitely non-irritating evoked slight, barely perceptible, erythema in 2 out of 10 control animals. In the treatment group 55 percent of the animals had irritant effects.

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The second challenge caused no primary irritation and cannot be used to define a sensitizing effect.

Conclusion:

Trifluralin may be a potential sensitizer but further testing is needed.

Classification: Supplementary; there was no positive control.

Reviewed by: Stephanie P. April, Ph.D. Stephanie April

Date: ~~5/6/86~~ 6/5/86 SPA reviewed date inadvertently

Secondary Reviewer: Marcia Van Gemert, Ph.D. M Van Gemert 6.9.86

Date: rec'd by me 6.5.86

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005898

DATA EVALUATION REPORT

Citation:

Test for Mutagenicity in Bacteria Strains in the Absence and Presence of a Liver Preparation (Ames Test), Dr. Engelbart and M. Scheerer, Hoechst Arbeitsgrupps Molekularbiologie, November 30, 1979; Study No. 74/79.

Materials:

Test Substance: HOE 38474 OH AT204, % purity not given

Organism Tested: Salmonella typhimurium strains TA98$^+$, TA100$^+$, TA1537$^+$, TA1538$^+$, and TA1535. Sprague Dawley rats were used but not further described.

Methods:

The methods used were according to Ames, B.N., McCann, J. and Yamasaki, E. "The methods for detecting carcinogens and mutagens with the Salmonella/Mammalian-Microsome Mutagenicity Test." Mutation Res. 31:347-364 (1975).

The bacterial test strains were maintained at -80 °C and recultivated in fresh media for testing. The Sprague Dawley rat was used as a source of the S-9 fraction of microsomes from liver homogenates. Five days prior to killing the rats 500 mg/kg PCB as Aroclor 1254 in corn oil (200 mg/mL i.p.) was used for liver enzyme induction. Aliquots of the S-9 liver homogenate fraction are used in a mix. This mix is tested with positive and negative mutagens to evaluate its reactivity.

The range of concentrations of test material was from 4 µg to 10,000 µg/plate with and without S-9 fraction activation using 8 plates per dose. The plates were incubated for 48 hours at 37 °C prior to counting the number of revertant colonies. Control plates (DMSO) were used to estimate the spontaneous mutation rate.

The positive control substances were 9-aminocridine (100 µg/plate) for TA1535, methyl-hydrazone derivative (5 µg/plate) for TA98, TA1538, and TA100 and Streptozotocin (10 µg/plate) for TA1537 with and without activation.

Results:

The following results were tabulated in the report:

Compound in test: HOE 38474 OH AT 204

Compound	µg/plate	S-9 Mix	Revertant colonies/plate TA1537	TA98	TA1538	TA100	TA1535
Control (DMSO)	0	+	4	30	12	89	7
			5	31	15	90	9
			7	37	15	95	20
			9	60	21	107	22
	10000	+	5	30	8	92	10
			5	33	11	95	13
			6	39	20	97	14
			12	48	22	100	19
	2500	+	6	29	12	64	9
			6	34	17	78	15
			7	42	20	81	18
			8	47	23	87	19
	500	+	5	23	10	81	10
			6	27	11	86	15
			7	28	13	89	15
			8	29	16	108	20
	100	+	4	36	11	96	12
			5	36	13	103	13
			7	46	13	110	16
			8	48	14	111	21
	20	+	4	24	13	73	10
			5	35	15	87	13
			7	42	20	108	13
			9	46	30	114	15
	4	+	5	32	8	78	10
			6	35	13	80	12
			14	38	21	102	14
			14	44	25	124	15
Positive controls							
2-Aminoanthracene	5	-	11	11	7	63	13
			18	23	8	90	14
2-Aminoanthracene	5	+	488	4200	4300	2700	144
			607	5000	4400	2900	162

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Compound in test: HOE 38474 OH AT 204

Compound	µg/plate	S-9 Mix	Revertant colonies/plate TA1537	TA98	TA1538	TA100	TA1535
Control (DMSO)	0	-	15	17	8	120	15
			15	25	10	128	18
			16	30	10	133	20
			18	35	12	136	21
	10000	-	10	17	8	101	21
			16	23	8	120	22
			17	24	8	125	23
			17	30	11	131	27
	2500	-	9	24	8	100	16
			10	31	11	109	20
			12	36	11	138	20
			13	39	12	136	25
	500	-	8	20	7	99	15
			9	26	9	112	17
			10	29	10	124	21
			16	31	14	129	21
	100	-	12	20	6	95	17
			13	26	10	113	18
			15	31	14	115	19
			15	32	14	122	23
	20	-	13	21	7	113	17
			14	25	9	115	21
			14	28	10	134	24
			17	29	10	136	33
	4	-	7	15	8	113	11
			8	22	9	125	20
			12	26	12	129	25
			17	28	15	136	25
Positive controls							
9-Aminoacridine	100	-	> 5000				
			> 5000				
Methyl-hydrazone Derivative	5	-		3300	2800	2800	
				3800	3000	3400	
Streptozotocin	10	-					> 5000
							> 5000

Conclusion:

The results clearly show that trifluralin was negative for mutagenic activity in S. typhimurium strains at doses up to 10,000 ug/plate. Trifluralin in the five bacterial strains of S. typhimurium used showed no mutagenic activity with or without activation.

Classification: Acceptable.

Reviewed by: Stephanie P. April, Ph.D. Stephanie April

Date: ~~5/6/86~~ 6/5/86 reviewed date inadvertently left

Secondary Reviewer: Marcia Van Gemert, Ph.D. M. Van Gemert 6.9.86
rec'd by me 6.5.86

Date:

DATA EVALUATION RECORD

005898

Citation:

Study of the capacity of the test article HOE 88474 OH AT 208 to induce "unscheduled DNA synthesis" in cultured HeLa cells. Angelo Mondino and Gianpaolo Berruto, RBM, Caseila Postale, Italy for Hoechst, No. A24439 June 9, 1982.

Materials:

Substance Tested: Trifluralin, technical (HOE 38474 OH AT208), a bright orange cystalline powder Certificate of Analysis No. 01365, 98.3%.

Cell Line: HeLa cells from Flow Lab, no passage number given.

Control Substances: Cyclophosphamide (CP) (Endoxan Asta Werk Batch No. 0391 Composition 69% CP and 31% NaCl).

Methylmethanesulfonate (MMS) (Merck Batch No. 8143447 d = 1.3).

Dimethylsulfoxide (DMSO) Merck, Batch No. 1107137 d = 1.1 in Endoxan.

Hydroxyurea (HU): 10 mM Sigma, 45.6 mg dissolved in 60 ml sterile medium fresh prior to use.

Tritiated thymidine (3H-Td3) - 25 ul are equivalent to Ci/ml.

Solutions and concentration of the test article

The test article HOE 38474 was dissolved in DMSO and further diluted to obtain the concentrations to be tested.

Reference standard solutions and concentrations

The reference standards were MMS for the test without metabolic activation and cyclophosphamide for the test with metabolic activation.

MMS was dissolved in DMSO to obtain the concentration of 10 mM. Endoxan was dissolved in DMSO to obtain the concentration of 14.2 mM of CP.

Preparation of S9 Mix for Metabolic Activation:

Adult male Sprague Dawley rats purchased from the Charles River Company (Calco) received a single intraperitoneal administration of an Aroclor 1254 solution in corn oil (200 mg/mL) at the dose of 500 mg/kg (2.5 mL/kg).

On the fifth day thereafter, the animals were sacrificed and the liver of each removed. The livers were homogenized with 0.15M KCl (3 mL per g of liver) for 30 seconds at 4 °c in three 10-second bursts.

The homogenate was centrifuged for 10' at 9,000 x g in a Sorvall refrigerated supercentrifuge. The supernatant was divided into fractions and deep frozen at -70 °C. The supernatant was assayed for protein concentration by the biuret method and for its activation capacity using cyclophosphamide with Salmonella typhimurium strains TA 1538, TA 98 and TA 100.

An adequate amount of S9 Mix was prepared immediately in an ice cold bath before use to have the following composition:

0.36 mL of HBSS
0.5 mL of liver microsomal enzymes (S9)
40 uL of $MgCl_2$ 20 mM
3.94 mg of NADP
6.08 mg of glucose-6-phosphate

Methods:

The dosage levels of trifluralin tested were 50, 100 and 500 ug/ml of incubation mixture both in the absence and presence of S9 mix. The report states that a preliminary test indicated that trifluralin was cytotoxic at 1000 ug/ml; however, the data were not included in this report. For each dose of the test article, the negative control (DMSO) and the positive control reference mutagens, six incubation wells (3 with hydroxyurea and 3 without) were incubated at 37°C for 24 hours. Each incubation well contained $2X10^5$ cells and 1 ml of medium. The hydroxyurea was used to inhibit replicative (S-phase) DNA synthesis so that mainly UDS could be scored.

The samples were assayed in a scintillation counter to compare the labelled tritiated thymidine incorporation in the cells of the various expermental groups. The test article is considered genotoxic if it induced a statistically significant response with (a dose-related correlation, i.e. stimulation of unscheduled DNA synthesis in cultured HeLa cells.

Results:

The mean cpm as values (average of 3 wells per treatment) as given in this report for this DNA repair assay on the test material, negative and positive control substances are reorganized by the reviewer and presented as follows:

Substance	Conc.	Mean CPM x 10^2 + SE Without Activation: Without HU'	T/C	With HU'	With Activation: Without HU'	T/C[2]	With HU'
DMSO	0.1 ml/ml	265.4 ± 23	-	8.22 ± 0.64	250.4 ± 42.3	_	7.78 ± 0.68
HOE 38474	50 ug/ml	165.6 ± 16.8	0.62	7.57 ± 0.51	175.6 ± 10.4	0.70	7.42 ± 0.64
HOE 38474	100 ug/ml	130.2 ± 11.8	0.49	7.15 ± 0.64	163.1 ± 29.4	0.65	5.45 ± 1.01
HOE 38474	500 ug/ml	163.8 ± 34.7	0.61	6.75 ± 1.35	150.3 ± 11.9	0.60	6.11 ± 0.51
MMS	1 mM	153.4 ± 16.6	0.57	17.67 ± 1.18*	-	-	-
CP	1.42 mM	-	-	-	215 ± 32.0	0.86	14.69 ± 1.81**

1 HU = Hydroxyurea

2 $T/C = \frac{\text{test material or positive control}}{\text{negative control}}$

* $p < 0.01$ (Student Method -no ref.)

** $p < 0.05$ (Student Method - no ref.)

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As is shown in the data above the incorporation of tritiated thymidine into the cells incubated with the various concentrations of the test material plus hydroxyurea with or without metabolic activation was not statistically different from that occurring in the cells with DMSO (negative control solvent) plus hydroxyurea.

In the cells incubated with the positive controls MMS with hydroxy urea without metabolic activation and the cells incubated with CP plus hydroxyurea with metabolic activation there was a statistically significant difference from negative control in the incorporation of tritiated thymidine in cultured (HeLa) human cells.

Discussion:

There was no testing done on nor was any data given on a cytotoxic dose level of the test material. A statistical method was indicated and p values given but the method used was not referenced.

Conclusion:

As was concluded by the laboratory in this test HOE 38474 OH AT208 did not induce statistically significant increases in incorporation of tritiated thymidine in the presence of hydroxyurea in cultured human cells (HeLa) with or without metabolic activation at doses up to 500 ug/mL.

The laboratory concluded that HOE 38474 OH AT208 in this study did not induce UDS (increased cell repair processes).

Classification:

Unacceptible for various reasons such as: (1) not tested to cytotoxic levels (2) no data from preliminary tests (3) no repeat test.

Reviewed by: Stephanie P. April, Ph.D. Stephanie P. April

Date: 7-2-86

Secondary Reviewer: Marcia Van Gemert, Ph.D.

Date: M. van Gemert 7.8.86

DATA EVALUATION RECORD 005898

Citation: Trifluralin (HOE 38474 OH AT208) Study of the test article for mutagenicity in the micronucleus test following oral administration to NMRI mice, Dr. Leist, Dr. Weigand, Prof. Kramer, Hoechst, Germany. Report No. 285/81, May 26, 1981

Materials:

Test Substance: HOE 38474 OH AT208, Hoechst AG, orange red crystals, Analysis No. 01365 was used in sesame oil. The purity of the substance was not given.

Animals: NMRI mice from age 7 to 12 weeks of Hoe NMRKF (SPF71) strain from Hoechst breeding colony of Pharma Forschung Toxikologie, Kastengrund, Hattersheim were used. The males weighed from 31 to 40 g and females from 23 to 28 g.

Positive Control: Cyclophospamide (Endoxan) Batch 9364 was prepared as a solution in distilled water as 100 mg/5 mL H_2O. Two mL of the 2 percent solution were mixed with 6 mL distilled water.

Methods:

Each treatment group contained five male and five female mice. The five groups used were administered 0, 25, 250, and 2500 mg/kg test substance or 100 mg/kg cyclophosphamide twice orally by gavage at a 24-hour interval. Six hours after the second dose the animals were sacrificed. The report states that 2500 mg/kg was found to be the TD but the data for this preliminary finding was not submitted nor was the toxicity described. Bone marrow was removed from the femur for preparaton of stained slides. The ratio of juvenile forms to normocytes and the number of normocytes with micronucli was determined from the examination of 2000 polychromatic erythrocytes per animal.

The method of binomial increase was used to compare experimental to control values for (1) the number of micronucleated polyhromatic erythrocytes per 2000 pcels and (2) the number of micronucleated normocytes per 1000 normocytes. This was done at the 90% significance level. The ratio of polychromatic to normochromatic erythrocytes in male and female eated animals was compared to controls by the method of Nemeny, Dunnett to test for sex specific deviations.

005898

It was stated that there was no statistically significant difference from control animals in treated animals for the number of micronucleated polychromatic erythrocytes or normocytes in this study. Trifluralin treated animals were not found to have a change of ratio of polychromatic erythrocytes to normocytes in this experiment, but again only individual animal data were given.

The positive control substance cyclophosphamide was found to induce a significant increase in the number of micronuleated polychromatic erythrocytes. In positive control group there was also a lower ratio of immature cells to mature cells.

Discussion:

Without demonstration of levels of trifluralin in the bone marrow at the time of sacrifice there was no evidence of it reaching the site of action however, the HDT had orange urine indicating absorption of trifluralin. A limit dose was given (2 x 0, 25, 250 and 2500 mg/kg) times 2 (24 hours apart) to reach a total of 0, 50, 500 and 5000 mg/kg even though no clinical toxicity or cytotoxicity was reported.

Conclusion:

The registrant indicated no evidence of a mutagenic effects and Toxicology Branch concurs.

Classification:

Acceptable.

Reviewed by: Stephanie P. April, Ph.D.

SPA

Date: 6/16/86

Secondary Reviewer: Marcia Van Gemert, Ph.D.

M. VanGemert 7.1.86

Date:

DATA EVALUATION RECORD 005898

Citation: Trifluralin technical - Code: HOE 38474 OHZD99002, Dominant Lethal Test for Determination of Mutagenic Effect in Male NMRI-Mice after Oral Administration Dr. Horstmann, Dr. Weigand, Prof. Kramer, Hoechst, Report No. 84.0763. November 16, 1984, Study No. G4Mo425.

Materials:

Test Substance: Trifluralin technical 98.3% ai, Code HOE 38474 OHZD99002, Certificate of Analysis No. 02404, orange-red crystals from Hoechst.

Positive Control: Cyclophosphamide (Endoxan) Asta, Bielefeld FRG; 100 mg vials with dry substance composition: 106.9 mg cyclophosphamide with H_2O and 45 mg Naci, equivalent to 100 mg cyclophosphamide.

Animals: 150 male and virgin female NMRI mice from Hoechst approximately 8 weeks of age weighing 33 ± 2.37 g. Colony (strain HOE: NMRKf (SPF71).

Methods:

The test material was dissolved in 5 ml sesame oil and administered by gavage daily for 5 consecutive days to groups of 30 male NMRI-mice at 10, 100, and 1000 mg/kg body weight per day. Negative and positive groups were administered daily doses of sesame oil (5 ml/kg) or 20 mg/kg cyclophosphamide in a 0.18 percent NaCl solution for 5 days. The day after treatment ended the males were mated for 4 days to virgin females (1:1) of the same breed. Fresh females were offerred treated males for 13 subsequent 4-day periods. On day 16 following each mating period the females were sacrificed and the number of live and dead implantations were recorded.

The behavior and general condition of the male and female animals were checked daily. The body weights of the males were recorded before first treatment, and before and after each mating interval. For the females the body weight was determined before each mating interval.

Results:

There were no adverse behavioral responses or clinical effects in the treated males. Nontreatment related transient dermal abrasions in the testicles of the male animals were observed and probably due to the frequency of mating. Two positive control (cyclophosphamide) males died on test and were excluded from the data evaluation.

There was no treatment related effect on the body weight gain in the males on test. The high dose (1000 mg/kg per day) did cause deep yellow discoloration of the urine indicating absorption of trifluralin.

Some females were excluded from analysis of results by unexplained spontaneous death (1 in the negative control), uterine pathology (1 in negative control, 4 in high dose and 1 in positive control group) and accidental preexperimental mating in transport (2 animals). The uterine pathology was femur uterine horns with opaque and thickaned serosa.

Placental fusions of two or three fetuses were seen in 5 females was enlargement to twice the size of the pregnant uterus and filled with clear fluid. The remaining uterine problem was fused uterine horns with opaque and thickened serosa. It was stated in the report that the test material did not decrease the pregnancy rate. The number of preimplantation or post-implantation losses were not significant according to the report since all values in treated groups were within the range of the negative control group.

Conclusion:

Trifluralin in this study did not induce dominant lethal mutations in the male mouse administered 5 consecutive doses of 10, 100 and 1000 mg/kg. The positive control substance increased the number of postimplantation fetal losses in the postmeiotic stages of male germ cells, indicating the sensitivity of this strain to respond.

Classification:

Acceptable.

Reviewed by: Stephanie P. April, Ph.D.
SPA

Date: 6/14/86

Secondary Reviewer: Marcia Van Gemert, Ph.D.
M. VanGemert 7.1.86

Date:

DATA EVALUATION REPORT 005898

Citation: Fumero and Berruto, In Vivo Study of Chromosome Aberration in the Chinese Hamster Induced by the Test Article HOE38474 OH AT208 Administered by Oral Route, May 31, 1982, RBM (Institute Di Recherche Biomediche), Study No. M373.

Materials:

Test Materials: HOE38474 OH AT208 (Batch No. is included in Code) as a bright orange crystalline powder. Certificate of Analysis No. 01365, November 24, 1980, 98.3% pure. Corn oil was used as the negative control and the reference mutagen Mitomycin C (Kyowa Batch No. 816 ASI) 10 mg/kg by intraperitoneal route was used as a positive control.

Animals: Adult male Chinese hamsters from Bantin and Kingman, Ltd., Grimston, Aldbrough Hull, United Kingdom, weighing 27 to 38 g.

Diet: Charles River 4RF22

Methods:

The test article was suspended in 5 mL corn oil at 45, 90, and 180 mg/mL for oral dosage by gavage at levels of 450, 900, and 1800 mg/kg twice, 24 hr. apart four animals per dosage group. These doses were stated to be based on a range-finding experiment and sponsor-supplied toxicological data which were not included in this report. Four animals received corn oil by gavage and four animals received a single dose of Mitomycin C i.p. (10 mg/kg). Six 6 hours after the second administration of test or control material all experimental animals received i.p. colchicine (C. Erba, Batch No. 7799 8D1537E), an antimitotic agent, at 6 mg/kg (10 mL/kg). The animals were killed by cervical dislocation an hour after administration of colchicine although two hours is recommended. Bone marrow was recovered from the femurs and suspended in Hanks' solution to 10 mL then centrifuged at 1000X g. The supernatant was discarded and the cells were washed with KCl and incubated for 15 minutes. The centrifugation, suspension, etc., was repeated several times and the final pellet was suspended in cold methanol -acetic acid and placed on microscope slides (4 per animal). The slides were stained and processed prior to reading at 1250X magnification. Approximately one hundred metaphases per animal were analyzed for aberrations according to the report.

005808

Statistical comparisons of the percent aberrations in each dose group to the percent in the control group were made using the Chi Square method (Snedecor, G.W. Statistical Methods, Iowa State College Piece, Ames, Iowa (5th Edition 1959) 228 and dose effect linear regression (no reference given). The total number of aberrations with and without gaps was recorded since gaps are not considered clear indications of genetic damage and could be methodological artifacts and not considered as aberrations. The gaps included were not defined.

The aberrations found are classified in this report as follows:

Fragment

Any piece of supernumerary chromatid material, free or displaced, in association or not with a parent chromatid. Minute fragments are classified as fragments.

Gap

An achromatic lesion that appears as a nonstaining region of one chromatid arm, the proximal and distal portions of which remain aligned. If the gap involves both chromatid arms in the same position it is defined as an isochromatid gap (isolocus gap).

Break

A lesion involving any separation or discontinuity of a chromatid piece with consequent derangement of the axial integrity and the possibility of the presence or the absence of the chromatid fragment that originated.

Exchange

Exchanges can be of two types: intrachanges, when the damage occurs within one chromosome and involves the internal rearrangement within the same chromosome, and interchanges, when the damage concerns two different chromosomes and the rearrangements occur between the two chromosomes: these can be of asymmetrical type when the two chromosomes involved have no symmetrical plan and of symmetrical type when they do.

Damaged metaphases

Metaphases that display appropriate chromosome distribution but contain several different types of damage not easily or individually identifiable.

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Results:

The data indicate that the positive control substance Mitomycin C induced statistically significant chromosome aberrations including and exc'uding gaps.

This table is compiled from the report by the reviewer.

Group	Number Animals	Tot. Aberrations including gaps	Tot. Aberrations excluding gaps	Breaks
Corn oil (negative control)	4	3	1	2
HOE 38474 OH AT203 450 mg/kg	4	1	0	0
HOE 38474 OH AT 203 900 mg/kg	4	0	3	0
HOE 38474 OH AT 203 1800 mg/kg	4	0	1	0
Mitomycinc (10 mg/kg)	4	116	76	9

There were no fragments or intrachanges or asymetric or symmetric interchanges in any of the test material or negative contorl groups. In the positive control group there were 25 fragments, 2 intrachanges, 41 symmetric interchanges and no asymmetri interchanges.

The submitted results indicated that no test dosage group had chromosome aberrations including and excluding breaks and gaps that were statistically different from control. According to the report breaks and gaps may be hereditary genetic damage or artifacts of the method and by themselves are not necessarily genetic damage from the material being tested.

Discussion:

The timing for sacrifice (6 hours after a second dose 24 hr) did not "account for (1) the possibility of chromasome damage after the first dose (within the 12-24 hr. cell cycle), and damaged cells incapable of reproducing (2) insufficient to sample damage throughout the second cell cycle, which would have required sampling at 12, 24 and perhaps 48 hr. post-dosing. Evidence for the possibility of significant mitotic delay was not given in this study nor were target tissue cytotoxicity.

There were no clinical effects ascribable to the test substance. An unexplained low death, occurred in the low- dose group.

Because of the missing information indicated above, the study should be repeated administering the test material and positive control i.p. There should be a high dose which is an MTD producing clinical toxicity and/or evidence of cytotoxicity at the target tissue.

Conclusion:

The study is unacceptable (1) because the time of sacrifice did not acccount for mitotic delay (2) cytotoxicity was not evident nor was clinical toxicity (3) the experimental data requires repetition to arrive on a conclusion.

Classification: Unacceptable.

Reviewed by: Stephanie P. April, Ph.D. SPA

Date: 6/16/86

Secondary Reviewer: Marcia Van Gemert, Ph.D.

Date: M. van Gemert 7.1.86

DATA EVALUATION REPORT

005898

Study Type: Mutagenicity - Gene mutation in yeast

Citation: Study of the Mutagenic Activity "In Vitro" of the compound HOE 38474 OH AT 208 with Schizosaccharomyces Pombe, RBM, Casella Postale, Italy, for Hoechst, Report No. A24391, May 27, 1982.

Materials:

Test Substance: HOE 38474 OH AT 208, a bright orange crystalline powder. Certificate of Analysis No. 01385, November 24, 1980, 98.3% pure, physically and chemically stable at 25° C and -5° C for more than 2 years according to the sponsor who also postulated 2 to 3 hours stability in DMSO.

Test System: A haploid mutant yeast strain Schizosaccharomyces pombe (SP ade 6-60/rad 10-198, h-) was originally supplied by "Laboratorio di Mutagenesi e Differenziamento" of the CNR (Pisa, Italy).

Positive Control Substances:

1. MMS (84.5 mg/L) form Merck Batch No. 8143447 d=1.3 wasused without metabolic activation.

2. DMNA (125 mg/L) Merck Batch No. 27283 d=1 was used with metabolic activation.

Negative Control Substance:

DMSO (27.5 mg/mL), Merck Batch No. 1107137 d=1.1

Methods:

The test material was used at 250, 500, and 1000 mg/L based upon a preliminary test where the report states that at 1000 mg/L the HOE 38474 OH AT 208 precipitates during incubation for 4 hours at 35° C. The results of this preliminary test are not given. The test material in varying concentrations was added to incubation media which was added to yeast on agar plates. Two positive control materials, one needing metabolic activatin and one not needing metabolic activation, as well as a negative control, the vehicle, were also tested.

The metabolic activation was accomplished by adding an S9 mix, consisting of the microsomal supernatant from the liver of a Sprague-Dawley rat which had been activated by i.p. Aroclor, plus co-factors (G-6-P/NADP).

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The mutation frequency is measured by counting mutated white colonies and dividing by the total number of colonies. This assay measures forward mutation induced in one of five loci preceding the 6th of the 10 genes controlling the adenine biosynthetic pathway in an adenine free medium. The adenine-free medium causes the strain of yeast to accumulate red pigment. The red pigment fails to accumulate when one of the five loci mutates so the mutated colonies are white.

Results:

The following tables indicate that in the "in vitro" test with or without metabolic activation the percent survival of the yeast with varying concentrations of test material (up to 1000 mg/L) did not differe significantly from the yeast with vehicle control. MMS, a positive control substance used without metabolic activation, did elicity a greatly reduced relative survival rate of the yeast while DMNA did so only to a lesser extent.

There was no statistically significant increase in the frequency of mutation for any concentration of HOE 38474 OH AT 208 that was tested with or without metabolic activation in comparison to the yeast with the negative control vehicle. The positive mutagen control substances were found to produce a statistically significant increase ($p < 0.001$) in mutation frequency in comparison to the vehicle control.

A dose-related particle precipitation of the test article was reported, but data were not provided in this report.

TABLE 1.

Mutagenicity study of the test article HOE 38474 OH AT 208 with Schizosaccharomyces pombe. Results of the "in vitro" test without metabolic activation.

Substance	Dose mg/L	Relative survival %	Mutants	Number colonies	Frequency $.10^{-4}$
Control	0	100	6	39340	1.52
HOE 38474	250	96	6	38780	1.54 (N.S.)
HOE 38474	500	93	4	37940	1.05 (N.S.)
HOE 38474	1000	93	5	37800	1.32 (N.S.)
MMS	84.5	30	36	12320	29.22*

* $p < 0.001$

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TABLE 2.

Mutagenicity study of the test article HOE 38474 OH AT 208 with Schizosaccharomyces pombe. Resuits of the "in vitro" test with metabolic activation.

Substance	Dose mg/L	Relative survival %	Mutants	Number colonies	Frequency $.10^{-4}$
Control	0	100	8	54320	1.47 (N.S.)
HOE 38474	250	97	8	52640	1.51 (N.S.)
HOE 38474	500	97	5	48620	1.02 (N.S.)
HOE 38474	1000	95	7	51800	1.35 (N.S.)
DMNA	125	88	26	44460	5.84*

* $p < 0.001$

Discussion:

The report mentions preliminary data from tests to arrive at the doses to be used for this study without submitting the data. In this preliminary test there was supposed to be precipitation of the test article which was proportional to the dose. These data were also not supplied.

The results are furnished for the percent survival of the yeast in each group, but the time of survival for these groups is not given and is required.

Conclusion:

The data provided appear to indicate that trifluralin does not induce gene mutation in S. pombe, at the limit of solubility in standard culture medium. The study is provisionally acceptable pending receipt of the data indicated above.

Core classification: Supplementary pending receipt of data

Reviewed by: Stephanie J. Lewis, Ph.D
7/3/87

Secondary Reviewer: M. Lewis Jarvis 4/28/87

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

005898

EPA: 68-02-4225
DYNAMAC No. 1-076A-1
August 13, 1986

005898

DATA EVALUATION RECORD

TRIFLURALIN

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Baeder, Weigand, and Kramer. Hoe 38474-active ingredient, testing for embryotoxicity in Wistar rats following oral administration. (Unpublished study No. A27236 prepared and submitted by Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie, Frankfurt, West Germany; dated October 18, 1983.) Accession No. 258993.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. C. Felkner

Date: 8-13-86

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005808

1. CHEMICAL: Trifluralin; Hoe 38474; 1,1,1-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.

2. TEST MATERIAL: Hoe 38474 OH AT 210, serial No. 10964-Op. 200/81, was a reddish-orange powder containing 99% active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Baeder, Weigand, and Kramer. Hoe 38474-active ingredient, testing for embryotoxicity in Wistar rats following oral administration. (Unpublished study No. A27236 prepared and submitted by Hoechst Aktiengesellschaft, Pharma Forschung Toxikologie, Frankfurt, West Germany; dated October 18, 1983.) Accession No. 258993.

5. REVIEWED BY:

Michael Narotsky, B.A.
Principal Reviewer
Dynamac Corporation

Signature: M. Narotsky
Date: Aug 13 '86

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 13 August '86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 8-13-86

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. The NOEL and LOEL for maternal toxicity are 100 and 500 mg/kg, respectively, based on one death, clinical observations, and decreased food consumption seen at 500 mg/kg.

The NOEL for developmental toxicity could not be established due to indications of reduced skeletal maturity and increased vascular fragility in fetuses at 20 mg/kg, the lowest dose level tested. Additional developmental effects were total litter resorption shortly after implantation at 100 mg/kg and reduced fetal weights and lengths and increased resorption rates at 500 mg/kg.

B. Since a NOEL for developmental toxicity was not determined, this study was classified Core Supplementary.

8. RECOMMENDATIONS:

In the event that further work is conducted, it is recommended that:

1. Lower dose levels be used in order to demonstrate a NOEL for developmental toxicity.

2. Results of chemical analyses on the concentrations and stability of the test material in the vehicle be submitted.

3. Individual maternal clinical signs, body weight, food consumption, and organ weight data be reported.

4. Individual fetal data be presented.

5. Fetal examination findings be summarized on a litter basis, and that they be statistically evaluated.

6. Historical control data for fetal abnormalities be submitted.

9. BACKGROUND:

Preliminary tests were conducted on pregnant Wistar rats dosed with trifluralin on gestation days (GD) 7-16. Doses of 40, 125, and 400 mg/kg were tolerated without complications by both dams and embryos. At 630 mg/kg, piloerection, decreased maternal body weight gain and food intake, and reduced fetal weights were present. Maternal deaths and reduced fetal weights were evident at 1000 mg/kg. Yellow discoloration of fatty tissue was present at all dose levels and reddish-orange urine was noted at doses of 125 mg/kg and greater.

Item 10--see footnote 1.

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[1] Only items appropriate to this DER have been included.

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11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

The test material (Hoe 38474 OH AT 210, serial No. 10964, Op. 200181) was described as a reddish-orange powder containing 99% active ingredient. Solutions were prepared every 4 days by mixing the test article with sesame oil t· produce dose levels of 0 (control), 20, 100, and 500 mg/kg.

Male Wistar rats (Hoe:WISKf strain, SPF71) from the Hoechst breeding colony were paired with females (approximately 70 days old). The day on which sperm was detected in the vaginal smears was designated GD 1. Approximately 24 mated females were assigned to each group and were dosed by gavage on GD 7-16. Maternal body weights and food consumption were recorded on GD 0, 7, 14, 17, and 21. The behavior and general health status of the rats were assessed daily.

The females were killed and grossly necropsied on GD 21. Heart, liver, kidneys, and spleen weights were recorded. Corpora lutea were counted and caesarean deliveries were performed. Uterine contents were examined for live fetuses, dead fetuses, and resorptions. Implantation sites were counted after staining the uterus with ammonium sulfide.

Fetuses were examined externally and their weights, crown-rump lengths, and placental weights were recorded. Fetal sex was determined at autopsy. Approximately one-half of the fetuses from each litter were dissected, eviscerated, and then stained with Alizarin Red S for skeletal examination. The remaining fetuses were fixed in Bouin's fluid for examination of visceral sections by Wilson's method.

In order to clarify results from the initial four-group study, a replicate study with animals dosed only at 500 mg/kg (with no concurrent control group) was conducted. The procedures used were apparently the same as for the initial four-group study except that, in the replicate study, the fetuses were not examined morphologically.

Maternal body weight, food consumption, organ weight, and fetal crown-rump length data were statistically evaluated using Dunnett's test. Fetal body weights and placental weights were analyzed according to Nemenyi/Dunnett. Corpora lutea and uterine findings were analyzed according to the method of Goodman. The data were also compared against historical control values.

B. Protocol: A study protocol was not provided.

12. REPORTED RESULTS.

A. Test Material: The authors indicated that the test material was stable in the dosing solutions for 3 days; however, no data regarding the concentration or stability of the test material in the vehicle were presented.

B. Maternal Effects: The only death reported in the study occurred in the replicated high-dose group after eight treatments. No clinical signs were noted for this animal on the previous day and advanced autolysis precluded a necropsy. No other information was reported.

No effects on behavior or general health were noted in the low- and mid-dose groups. At the high-dose level, however, all dams (both groups) had yellow or orange-yellow discoloration of the urine. Increased urinary excretion was observed in 16 animals of both high-dose groups. In addition, one dam of the initial high-dose group was markedly drowsy and had a blood-encrusted nose on GD 8 and 9. Bristled fur during the second half of the treatment period was observed in one dam of the initial high-dose group and in two dams of the replicate group. The only other reported finding was localized hair loss occurring sporadically in the control, low-, and initial high-dose groups.

The study authors reported that maternal body weight gains were comparable in all groups (Table 1). Food consumption was comparable to controls in the low- and mid-dose groups, but slightly decreased in both high-dose groups during the first week of treatment (Table 2).

Necropsies revealed yellow discoloration of fatty tissues in most dams receiving 100 and 500 mg/kg. Distention of one or both renal pelves was noted in one, one, two, and two dams in the low-, mid-, initial high-, and replicate-dose groups, respectively. The study authors reported that one of the affected high-dose dams had limpid fluid in the renal pelvis and another dam had light grey hollows on the kidney surface; one kidney was also enlarged with yellow calculi in the pelvis. Liver and spleen weights were significantly increased in dams receiving 500 mg/kg when compared to controls (Table 3).

C. Developmental Effects: Pregnancy rates were comparable in all groups; however, total litter resorption occurred shortly after implantation in one, four, and six dams of the mid-, initial high-, and replicate-dose groups, respectively. The number of corpora lutea and implantations per dam were comparable among all groups. Reduced litter sizes and correspondingly increased resorption rates were noted in both high-dose groups; the values were significantly different from controls in the initial high-dose group. In addition, nonsignificant reductions in fetal weight (Table 4) and length were noted in both high-dose groups. Placental weights were comparable in all groups.

TABLE 1. Mean Maternal Body Weights (g±SD) of Rats Dosed with Trifluralin

Dose Level (mg/kg)	No. of Dams	Gestation Day 0	7	14	17	21
0	20	195± 9	220±12	245±13	262±16	308±20
20	20	197± 8	223±13	253±15	271±19	322±24
100	19	190±11	218±12	241±14	258±14	306±19
500	16	198±13	227±13	242±21	258±19	304±33
500 (replicate)	13	189±14	219± 9	228± 9	239± 9	289±13

Dose Level (mg/kg)	Weight Gain[a]--Gestation Days 0-7	7-14	14-17	17-21	Total 0-21	Adjusted[d] 0-21
0	25	25	17	46	112±20[b]	69±13
20	26	30	18	51	125±21	78±17
100	28	23	17	48	116±13	73±11
500	29	15	16	46	105±29	66±18
500 (replicate)	30	9	11	50	100±16[c]	62±12

[a]Calculated by reviewers (except for Total Weight Gain); not statistically analyzed due to the absence of individual animal data.
[b]Reviewers' calculations indicate mean of 113.
[c]Reviewers' calculations indicate mean of 101.
[d]Adjusted Weight Gain = Total Weight Gain - [(mean fetal weight + mean placental weight) x No. live fetuses].

TABLE 2. Mean Maternal Food Consumption (g/100 g body weight/day ± SD) in Rats Dosed with Trifluralin

Dose Level (mg/kg)	Gestation Days			
	1-7	7-14	14-17	17-21
0	8.51±0.92	6.77±0.59	6.98±0.63	7.84±0.52
20	8.82±0.86	7.26±0.68	6.92±0.74	8.09±0.51
100	9.04±0.80	6.79±0.79	6.66±0.65	8.34±0.64
500	8.53±0.66	5.24±1.31	6.43±0.66*	8.55±0.63*
500 (replicate)	9.41±0.54*	4.90±0.60	6.64±1.22*	9.54±0.89*

*Significantly different from control value ($p<0.05$).

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TABLE 3. Mean Organ Weights (±SD) of Rats Dosed with Trifluralin

Dose Level (mg/kg)	No. of Dams	Body Weight (g)	Liver Weight (g)	Spleen Weight (g)
0	20	308±20	12.96±1.26	0.56±0.07
20	20	322±24	14.04±1.31*	0.63±0.11
100	19	306±19	13.66±1.08	0.63±0.13
500	16	304±33	15.22±1.73*	0.76±0.10*
500 (replicate)	13	289±13	14.82±1.17*	0.71±0.07*

*Significantly different from control value ($p<0.05$).

TABLE 4. Summary of Reproduction and Litter Data for Rats Dosed with Trifluralin

Dose Level (mg/kg)	Number of Females				Mean[b] per Litter				
	Mated	Pregnant	With Total Resorptions	With Live Fetuses	Corpora Lutea	Implantations	Resorptions	Live Fetuses	Fetal Weight (g)
0	23	20	0	20	13.2	12.0	0.25	11.8	3.24
20	20	20	0	20	13.4	13.0	0.30	12.7	3.26
100	22	20	1	19	13.7	12.6	0.74	11.8	3.19
500	24	20	4	16	14.6	13.1	2.31*	10.8*	3.09
500 (replicate)	21	20[a]	6	13	13.3	11.8	1.08	10.8	3.09

[a]Includes one dam that died.

[b]Means are based on litters with live fetuses.

*Significantly different from control value ($p<0.05$).

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Fetal examinations revealed hematomas (internal and external) or hemorrhage in the abdominal cavity in each of the dose groups.

The study authors stated that compound-related effects on skeletal development were evident in the high-dose group. Fetuses in this group had increased incidences of reduced ossification of the skull, ribs, vertebrae, and 5th metacarpal and also increased incidences of lumbar ribs and thickened, wavy, or bent ribs (Table 5). Shortened and bent scapulae, sometimes occurring in association with short humerus or bent basioccipital, was observed in one, zero, two (from two litters), and six fetuses (three litters) of the control, low-, mid-, and high-dose groups, respectively. The authors regarded the mid-dose findings to be spontaneous while the high-dose findings were associated with the reduced ossification, which was considered compound related.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the NOEL for maternal and embryonic/fetal toxicity is 20 mg/kg. Effects noted at 100 mg/kg were discoloration of maternal fat and, in one dam, the death of all embryos shortly after implantation.

At 500 mg/kg, the study authors also noted a single maternal death, clinical signs of intolerance, decreased food intake, increased excretion of yellow to orange-yellow urine, yellow discoloration of fatty tissues, and increased liver and spleen weights in the dams. Additional effects at 500 mg/kg were embryonic deaths shortly after implantation and fetuses with slightly retarded development frequently exhibiting skeletal variations and deformations. The test material was not considered teratogenic at the dose levels tested.

B. A quality assurance statement was signed and dated October 18, 1983.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material: Although the test material was reportedly stable in the dosing solutions for 3 days, no data were submitted for verification of the concentrations or stability of the test material in the vehicle.

Maternal Effects: We assessed that maternal body weights and weight gains did not indicate a toxic effect on the dams. Body weights and total weight gains were comparable for all groups (Table 1). Assessment of the total weight gains adjusted for fetal and placental weights suggested that decreases in litter size and fetal weights may have contributed to the slight decreases in weight gain noted during the dosing period at the high-dose level. Weight gains for each interval could not be statistically evaluated since individual body weights were not reported.

TABLE 5. Summary of Fetal Examination Findings

Dose Level (mg/kg)	No. of Fetuses Examined	Visceral Examination: No. of Fetuses Affected: Intra-Abdominal Hemorrhage	Hematoma: Cervical	Liver	Adrenal	Hindleg	Tail
0	114	0	0	0	0	0	0
20	122	1	0	1	0	1[a]	1[a]
100	108	1	0	0	1	1[a]	0
500	81	2	1	0	0	1	1[a]

Dose Level (mg/kg)	No. of Fetuses Examined	Skeletal Examination--Percentage of Fetuses Affected: Reduced Ossification: Skull	Vertebrae	Ribs	Thickened, Wavy, or Bent Ribs	Shortened, Bent Scapula	Lumbar Rib
0	122	26.2	0	0	3.3	0.8	36.1
20	132	39.4	0	0	6.8	0	29.5
100	117	28.2	0	0	10.3	1.7	37.6
500	92	47.8	7.6	10.9	34.8	6.5	50.0

[a]Noted in fetuses selected for skeletal examination.

Decreased food consumption (relative to body weight) was evident throughout the treatment period in dams receiving 500 mg/kg; the decreases were significantly different from controls on GD 14-17. After treatment termination, a significant compensatory increase was evident at the 500-mg/kg level (Table 2).

Increased urinary excretion, piloerection, a single death, and one dam with drowsiness in the high-dose groups indicated a toxic effect at 500 mg/kg. Increased spleen and liver weights at 500 mg/kg (Table 3) suggested a metabolic response to the compound rather than maternal toxicity. Yellow discoloration of fatty tissues, noted at 100 and 500 mg/kg, and yellow discoloration of the urine, noted at 500 mg/kg, were also not considered toxic effects.

Developmental Effects: The occurrences of total litter resorption shortly after implantation (Table 4) were considered compound related at the 100- and 500-mg/kg levels.

Uterine examinations of dams with live fetuses revealed increased resorption rates and, consequently, reduced litter sizes at 500 mg/kg (Table 4). Nonsignificant reductions in fetal weight and length also indicated developmental toxicity at 500 mg/kg.

Fetal examination findings, when evaluated with the litter (rather than the fetus) as the experimental unit, indicated mild reductions in skeletal maturity and slightly increased vascular fragility at all dose levels. Hematomas and hemorrhages were noted only in the dose groups; their combined litter incidence was significantly greater than controls in the low- and high-dose groups (Table 6). Increased incidences of thickened, wavy, or bent ribs occurred at all dose levels with a significant increase at the high-dose level. In addition, incidences of unossified 5th metacarpals were increased at all dose levels when compared to controls; the litter incidence was significantly increased at the low-dose level and the mean proportion per litter was significantly increased at the high-dose level. The reviewers also considered the nonsignificant increases in the incidences of shortened and bent scapulae to be compound related in the mid- and high-dose groups. This effect was more severe in the high-dose groups; some affected high-dose fetuses also had a shortened humerus or bent basioccipital.

Other findings consistent with decreased skeletal development were reductions in ossification of the vertebrae and/or ribs in the high-dose group (Table 5) and a nonsignificant dose-related decrease in the mean number of caudal vertebrae per litter at all dose levels (Table 6).

TABLE 6. Summary of Litter Incidances of Fetal Examination Findings

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Dose Level (mg/kg)	No. of Litters Examined	No. of Litters Affected[a]					
		Reduced Ossification of Skull	Lumbar Rib	Thickened, Wavy, or Bent Ribs	Shortened, Bent Scapula	Unossified 5th Metacarpal	Hematoma or Hemorrhage
0	20	14	15	3	1	12	0
20	20	16	16	7	0	17*	4*
100	19	16	16	7	2	15	3
500	16	13	14	13*	3	14	5*

Dose Level (mg/kg)	Mean No. Caudal Vertebrae per Litter[b]	Mean Proportion of Affected Fetuses per Litter[b]					
		Reduced Ossification of Skull	Lumbar Rib	Thickened, Wavy, or Bent Ribs	Shortened, Bent Scapula	Unossified 5th Metacarpal	Hematoma or Hemorrhage
0	1.86	0.28	0.38	0.04	0.01	0.32	0.00
20	1.71	0.40	0.28	0.06	0.00	0.49	0.02
100	1.67	0.29	0.37	0.11	0.02	0.47	0.01
500	1.62	0.43	0.49	0.31*	0.07	0.68*	0.03

[a] Compiled by reviewers and statistically analyzed using Fischer's Exact test.

[b] Compiled by reviewers and statistically analyzed using Analysis of Variance followed by Duncan's test for multiple comparisons.

*Significantly different from control value ($p \leq 0.05$).

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B. The reviewers' interpretation of maternal food consumption data differed slightly from that of the study authors. We considered high-dose food consumption to be decreased for the entire treatment period rather than just the first week of treatment. This was considered a minor difference since it did not alter the assessment of the NOEL for maternal toxicity.

The study authors' assessment of developmental effects at the high-dose level was generally similar to that of the reviewers. However, there are differences of interpretation at lower doses. Compound effects noted by the reviewers include shortened and bent scapulae and total litter resorption at the mid-dose level and deformed ribs, unossified 5th metacarpals, and hematomas or intra-abdominal blood at all doses. The study authors noted only total litter resorption as an effect at the mid-dose level.

C. The following deficiencies were noted:

1. Data regarding the concentration and stability of the test material in the vehicle were not submitted, thereby precluding the verification of the suitability of dosing preparations.

2. Individual maternal data were not presented for body weights, food consumption, clinical observations, necropsy observations, or organ weights. This deficiency precluded the correlation of developmental effects with maternal effects.

3. In their evaluation of fetal observations, the study authors did not combine incidences of related findings (e.g., hematomas in various locations), nor were the findings summarized on a litter basis or statistically analyzed.

4. Due to the presence of fetal effects at all dose levels tested, a NOEL for developmental toxicity could not be established.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 6-8.

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APPENDIX A

Materials and Methods

Trifluralin toxicology reviews

Page _____ is not included in this copy.

Pages 62 through 64 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

005898

EPA: 68-02-4225
DYNAMAC No. 1-076A-2
August 13, 1986

005898

DATA EVALUATION RECORD

TRIFLURALIN

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Becker, H. Embryotoxicity study with trifluralin-substance technical grade (Code: HOE 038474 OH ZD99 0002) in the rabbit (oral administration). (Unpublished study No. A29709 prepared by Research and Consulting Co. AG, Itingen, Switzerland, for Hoechst AG, Federal Republic of Germany; dated August 9, 1984.) Accession No. 258993.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 8-13-86

005898

1. CHEMICAL: Trifluralin; 1,1,1-trifluoro-2,6-N,N-dipropyl-p-toluidine.

2. TEST MATERIAL: Technical grade trifluralin (code: HOE 038474 OH ZD99 0002) was described as orange crystals containing 98.4 percent active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

4. STUDY IDENTIFICATION: Becker, H. Embryotoxicity study with trifluralin-substance technical grade (Code: HOE 038474 OH ZD99 0002) in the rabbit (oral administration). (Unpublished study No. A29709 prepared by Research and Consulting Co. AG, Itingen, Switzerland, for Hoechst AG, Federal Republic of Germany; dated August 9, 1984.) Accession No. 258993.

5. REVIEWED BY:

Michael Narotsky, B.A.
Principal Reviewer
Dynamac Corporation

Signature: Michael Narotsky
Date: Aug. 13, '86

Robin B. Phipps, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Robin B. Phipps
Date: August 13, 1986

6. APPROVED BY:

Guillermo Millicovsky, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: G. Millicovsky
Date: 13 August '86

Marcia Van Gemert, Ph.D.
EPA Reviewer and Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. The absence of toxic effects in this study, even at the highest dose level tested (60 mg/kg), precluded assessment of the LOEL for maternal and developmental toxicity of trifluralin.

B. The study was classified Core Supplementary due to the lack of toxic effects.

8. RECOMMENDATIONS:

In the event that further work is conducted, it is recommended that:

1. Higher dose levels be used to achieve some maternal toxicity as suggested by the USEPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation, November 1982.

2. Data from chemical analyses of dosing suspensions be reported.

3. The animals be randomized in a manner that ensures comparable initial body weights among all groups.

9. BACKGROUND: The study author indicated that in a preliminary study (RCC Project 032376) 30 mg/kg/day produced diarrhea. Toxic effects at 100 mg/kg/day were manifested by diarrhea and the death of one female. The author did not present further details on the methods or results of this preliminary study in the teratogenicity study report.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material used in this study was technical grade trifluralin and was described as orange crystals with a purity of 98.4 percent. Dosing mixtures of the test material and sesame oil were prepared daily to provide dose levels of 0 (control), 4, 16, and 60 mg/kg. The mixtures were administered by gavage at a volume of 2 mL/kg on gestation days (GD) 6-18; volumes were adjusted daily according to body weight.

2. Sixteen mated female hybrid chinchilla rabbits, SPF quality (KFM Kleintierfarm Madoerin AG, Switzerland), were randomly assigned to each of the four study groups. The rabbits were

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[1] Only items appropriate to this DER have been included.

4 to 6 months old at mating and weighed between 2302 and 3658 g. Animals were observed twice daily for clinical signs, health status, and mortality. Body weights were recorded daily from GD 0 (day of mating) until GD 28. Food consumption was recorded on GD 6, 11, 15, 19, 24, and 28.

3. On GD 28 the dams were killed by cervical dislocation and internal organs were examined grossly. Uteri of apparently nonpregnant females were placed in aqueous ammonium sulfide and examined for implantation sites. Gravid uterine weights were obtained to calculate corrected body weight gains. The number of corpora lutea and the number and uterine position of fetuses and resorptions were recorded.

   Fetuses were delivered by caesarean section, individually weighed, and examined for external abnormalities. The body cavities and organs of all fetuses were grossly examined and the sex of each fetus and any abnormal findings were recorded. The heart and kidneys of each fetus were fixed in 4% formalin, sectioned, and examined. Fetal heads were skinned, examined for cranial ossification, and fixed in a solution of trichloroacetic acid and formaldehyde, sectioned, and examined according to Wilson's method. Fetal trunks were cleared in a potassium hydroxide solution and stained with alizarin red (by a modification of Dawson's technique) for skeletal examination.

4. Analysis of variance was used to evaluate body weight, food consumption, organ weight, and reproductive data. Single treatment groups were compared against controls using Student's T-test. Reproduction data were evaluated using a Kruskall-Wallis analysis of variance (one-way) based on Wilcoxon ranks. Fisher's exact test for 2 x 2 tables was applied if the variables could be dichotomized without loss of information. Fetal sex ratios were evaluated using a 2 x 2 Chi-square analysis.

B. Protocol: A protocol was not provided in the study report.

12. REPORTED RESULTS:

A. Analysis of Test Material: The test material was reportedly stable in the vehicle under the conditions of use. However, no data on the stability or concentrations of the test material in the dosing mixtures were presented.

B. Maternal Data: None of the females died during the study, and no compound-related clinical signs were observed. One low-dose dam (4 mg/kg) had slight dyspnea on GD 10 and one high-dose dam (60 mg/kg) had slight diarrhea on GD 6-14; no other clinical signs were reported.

Although the females were randomly assigned to groups, the initial weights of the control dams were greater than in the dose groups. Consequently, control animals were heavier than dosed animals throughout gestation. Body weight gains and corrected weight changes, however, were comparable for all groups (Table 1).

The authors stated that food consumption was significantly reduced for the mid- and high-dose groups prior to compound administration when compared to controls. During and after the treatment period, however, food consumption data were comparable for all groups (Table 2).

Necropsies on GD 28 revealed only incidental findings. One low-dose dam had unilateral urinary tract agenesis and two, two, and one female of the low-, mid-, and high-dose groups, respectively, had kidneys with granulated surfaces, crateriform indentations, or grey-white foci. The study author reported that these findings are common in rabbits and were not considered to be compound related. No other necropsy data for the dams were reported.

C. Developmental Data: No compound-related differences between groups were evident in the uterine data. The incidence of preimplantation and postimplantation losses did not indicate a compound effect and litter sizes were comparable for all groups. In addition, fetal body weights were comparable for all groups (Table 3).

Findings from gross, skeletal, and visceral (body cavity, heart, kidney, and cephalic) examinations were incidental and not dose related (Table 4).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that under the conditions of this study, the NOEL for both the maternal and fetal organisms was greater than 60 mg/kg.

B. A quality assurance statement was signed and dated September 26, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Maternal Effects: All dams survived the experimental period. Clinical observations were noted in only one dam in each of the low- and high-dose groups. We regard these findings to be incidental.

TABLE 1. Mean Maternal Body Weights (g ± SD) of Rabbits Dosed with Trifluralin

Dose (mg/kg)	Gestation Day 0	6	19	28
0	3226±330	3403±331	3550±318	3700±270
4	2776±220	2996±245	3162±277	3357±287
16	2811±225	3019±236	3155±277	3358±264
60	2803±203	3007±235	31,3±194	3370±212

Dose (mg/kg)	Weight Gain--Days 0-6	6-19	19-28	6-28	Percent Corrected Weight Change[a]
0	177	147	150	297	-3.6±2.7
4	220	166	195	361	-1.0±3.2
16	208	136	203	339	-1.2±5.6
60	204	166	197	363	-1.5±4.9

[a]Corrected body weight change = (day 28 body weight) - (day 6 body weight) - (gravid uterine weight). Percentage is based on day 6 weight.

TABLE 2. Mean Food Consumption (g/animal/day ± SD) of Rabbits Dosed with Trifluralin

Dose (mg/kg)	Gestation Days					
	0-6	6-11	11-15	15-19	19-24	24-28
0	217±17	150±34[a]	172±41	190±47	202±31	165±27[b]
4	202±39	163±45	158±37	176±32	195±34	164±29
16	189±29*	146±53	152±45	156±60	194±37	174±37
60	192±35*	131±48	166±30	195±37	221±44	163±47

[a]Reviewers' calculations indicate mean of 151.

[b]Reviewers' calculations indicate mean of 166.

*Significantly different from control value ($p < 0.05$).

TABLE 3. Summary of Reproductive Data of Rabbits Dosed with Trifluralin

Dose (mg/kg)	No. Pregnant	Mean No. Corpora Lutea	Mean No. Total Implants	Percent Preimplantation Loss[a]	Percent Postimplantation Loss[b]	Mean No. Live Fetuses
0	14	10.0	8.8	12.1	9.8	7.9
4	16	9.3	7.8	16.2	5.6	7.3
16	16	9.2	8.2	10.9	13.7	7.1
60	15	8.8	8.3	5.3	8.0	7.7

Dose (mg/kg)	Mean ± SD (g) Fetal Weight Per Litter	Percent Male Fetuses[c]
0	33.4±3.4	45.9
4	35.4±4.4	49.6
16	35.0±3.1	45.1
60	34.0±3.0	53.0

[a] Preimplantation loss = $\frac{\text{(No. corpora lutea) - (No. implants)}}{\text{(No. corpora lutea)}}$

[b] Postimplantation loss = $\frac{\text{(No. implants) - (No. live fetuses)}}{\text{(No. implants)}}$

[c] Based on live fetuses.

TABLE 4. Summary of Fetal Examination Findings of Progeny from Rabbits Dosed with Trifluralin

Dose (mg/kg)	Fetuses (Litters) Examined	Fetuses (Litters) Affected								
		Scoliosis, Shortened Tail	Gastros-chisis	Unilateral Urinary Tract Agenesis	Unilateral Uterine Agenesis	Diaphrag-matic Hernia	Internal Hydro-cephaly	Dilated Renal Pelvis	Sternebral Variations	Fused Ribs
0	111 (14)	1	0	3 (1)	0	0	0	3 (3)	4 (4)	0
4	117 (16)	0	1	0	0	0	0	0	1	0
16	113 (16)	0	0	0	1	1	1	2 (2)	4 (4)	1
60	115 (15)	0	0	0	0	0	0	1	1	1

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Maternal body weights at mating (and throughout gestation) were heavier in the control group than in any of the dose groups. This initial difference in body weights is a potential confounding factor of the study and complicates the interpretation of the data. However, since body weight gains and corrected body weight gains were comparable in all groups (Table 1), we assess that this deficiency did not invalidate this study. No compound-related effects were evident in the food consumption data (Table 2).

Necropsy observations were limited to a congenital defect in a control dam and to kidney lesions in two, two, and one dam from the low-, mid-, and high-dose groups, respectively. We assess that similar kidney lesions are commonly seen in rabbits and their relatively low incidence in this study is not considered to be compound related.

Developmental Effects: No compound-related effects were evident from the uterine findings. Preimplantation and postimplantation losses revealed no adverse effects of the compound. In addition, litter sizes and fetal weights were also comparable in all groups (Table 3).

Based on available data on fetal examinations, we assess that the gross, visceral, and skeletal findings were incidental and not compound related (Table 4).

B. We agree with the study author's interpretation that no maternal or developmental toxicity was evident in this study.

C. The following deficiencies were noted in this study:

1. Chemical analyses were not presented, thereby precluding verification of the stability and the concentrations of the test material in the dosing preparations.

2. The reviewers regarded the initial weight differences between the control and dosed animals to reflect a deficiency in the randomization procedure. The animals could have been rerandomized prior to compound administration or a weight-stratified randomization scheme could have been used to allow a homogenous weight distribution between groups.

3. Since no toxicity was observed, the dose levels selected for this study were not high enough to establish the LOEL for either maternal or developmental effects.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-14 and 83.

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APPENDIX A

Materials and Methods

Trifluralin toxicology reviews

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Pages 76 through 82 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

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Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 4/22/87
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

005898

DATA EVALUATION REPORT

STUDY TYPE: Inhalation study in rats TOX. CHEM. NO.: 889

ACCESSION NUMBER: 258996 MRID NO.: ?

TEST MATERIAL: HOE 38474 OH AT 210

SYNONYMS: Trifluralin

STUDY NUMBER(S): 5488

SPONSOR: Hoechst Aktiengesellschaft

TESTING FACILITY: Research Consulting Co Ltd. Itingen Switzerland

TITLE OF REPORT: 30-day repeated dose inhalation toxicity study with HOE 38474OH A7 210 active ingredient (technical)

AUTHOR(S): L. Ullman

REPORT ISSUED: Feb. 12, 1982

CONCLUSIONS: Doses tested in nose-only inhalation study with exposure 6 hrs/day, 5 days/week were 100, 301 and 1006 mg/CBM. Effects were detected in group 4 showing signs of toxicity 6 hours after initial exposure. There was an increase in group 4 methemoglobin levels, however, the data were not presented to confirm or deny this statement. Total bilirubins were increased in the mid and high dose groups. The study stated that direct bilirubins were also elevated, but did not give the data to confirm this statement. Group 3 and 4 male and female absolute and relative liver weights were increased, and centrilobular hypertrophy of the liver was seen in high dose females and at all doses for males.

NOEL < 100 mg/CBM (LDT) based on centrilobular hypertrophy seen in males at all doses tested.

Classification: core-Supplementary, No macroscopic summary tables accompany the study text. Methemoglobin and direct bilirubin data are also missing from the study text.
Special Review Criteria (40 CFR 154.7)

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A. MATERIALS:

1. Test compound: Trifluralin, Description red solid, Batch # A201751, Purity 99.0%, contaminants: list in CBI appendix Stated to be stable in original container at +25°C or -5°C for 2 years.

2. Test animals: Species: rats, Strain: Wistar, KF_Han (outbred, SPF-quality) Age: 10 weeks, at study initiation Weight: Males- 175-180 gm., females- 171-177 gms. Source: Kleintierfarm, Nadderin, AG4414 Fuellinsdorf, Switzerland

B. STUDY DESIGN:

1. Animal assignment

15/sex/group were assigned to the following test groups: 10 animals/sex/group were assigned to the main study which ran for 30 days. 5 animals/sex/group were reserved for an additional 14 days recovery period.

Test Group	Nominal air	Conc'n determined gravimetrically mg/CBMx/S.D.	Conc'n determined chemically mg/CBMx/S.D.
1 Cont.	600 l air/hr		
2 Low (LDT)	1.0 ml/hr	100 ± 5	106 ± 19
3 Mid (MDT)	2.0 ml/hr	301 ± 8	307 ± 56
4 High(HDT)	10.0 ml/hr	1006 ± 23	1040 ± 139
5 Solvent	10 ml acetone/hr		

2. Exposure

Exposure (nose only) was for 6 hours/day, 5 days/week for a total of 22 exposures.

3. Animals received food (Kliba 24/343/1 Batch 56/81) and water ad libitum.

4. Statistics - The procedures utilized are on appended page 1.

5. Quality assurance statement was not given.

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C. METHODS AND RESULTS:

1. Observations

Animals were inspected daily for signs of toxicity and mortality. Appended pages 3 and 4 list the daily symptoms that were checked.

Results:
Toxicity: Group 4- after 6 hours on the first day of exposure and thereafter, showed slight dyspnoea and ruffled fur. These symptoms were continually seen just after exposure. No other groups showed signs of toxicity.

Mortality: No animals died during the duration of the test.

2. Body weight

Animals were weighed twice before starting the experiment, and twice weekly thereafter.

Results: Treated animal body weights were comparable to controls.

3. Food consumption and compound intake

Consumption was determined weekly throughout the entire test period. Food conversion was calculated according to the following formula:

$$MFC = \frac{\text{weekly food consumption}}{\text{body weight (gms)}} \times \frac{1000}{7}$$

Food consumption and conversion were measured only on 3 animals/sex/group.

Results: no effects were seen in food consumption. Group 4 females showed an increase in food conversion, However, the number of animals (3/group) makes this calculation useless.

4. Ophthalmalogical examinations

Performed once during the pretest phase and daily during the exposure and recovery periods.

Results: No treatment-related ocular changes were noted.

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5. Blood was collected from 10 animals/sex/group after 30 days between 7:00 and 9:30 AM. Checked parameters were measured.

a. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*		Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*		Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

Results:
The statistical analyses in appendix O and the study text state that both total and direct bilirubin levels in group 4 females were significantly elevated. However, there are no data presented for direct bilirubin levels in the tables, or study text. No other clinical chemistry parameters appear to be affected by treatment.

Table I

females:	Total Bilirubin ± S.D.
1.	3.9 ± 1.3
2.	5.1 ± 2.1
3.	4.1 ± 0.8
4.	6.3 ± 1.1 **
5.	3.8 ± 1.2

** = Significantly different from controls, $p < 0.01$

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b. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Heinz bodies
X	Thromboplastin time	X	Methemoglobin
X	Partial thromboplastin time		

Results: According to the study text there was a significant increase in methemoglobin formation in group 4 females. However, that information was not presented in the accompanying tables, and cannot be comfirmed.

6. Urinalysis were not performed.

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7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed for all animals killed at termination of exposure and recovery period.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	.Aorta*	XX	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*# D
X	.Esophagus*		.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary*
X	.Duodenum*	X	.Spleen*	X	Eyes (optic n.)*# A
X	.Jejunum*	X	.Thymus*		Glandular
	.Ileum*		Urogenital	XX	.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
	.Rectum*	XX	.Testes*†		.Parathyroids*††
XX	.Liver*†E	X	Epididymides	X	.Thyroids*††
	Gall bladder*#	X	Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*# C
	Respiratory	XX	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung* B			X	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

A- eye and contiguous Harderian glands examined microscopically
B- Examined microscopically with mainstem bronchi
C- examined microscopically with marrow
D- sciatic nerve
E- two lobes

Histopathological exams were done on all control and high dose animals, after 30 days of exposure. Also tissues of those animals of other groups where abnormal findings were noticed after macroscopic inspection were investigated. Lung, liver, spleen, bone marrow and kidneys were examined in rats of low, mid and recovery groups.

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a. Organ weight

Absolute weights: There was a significant dose-response relationship for liver. In males and females of groups 3 and 4 liver weights were significantly different from controls.

Relative Weights (organ/body weights, organ/brain weights)
Liver to body weight and brain weight ratios were significantly increased in groups 3 and 4 males and females compared with controls, with a significant trend apparent for both sexes.

TABLE II

Liver Weights

Absolute weights ± S.D.

Group	males	females
1	10.11 ± 1.13	7.09 ± 0.59
2	10.70 ± 1.21	7.79 ± 0.76
3	11.14 ± 1.14*	8.39 ± 0.72*
4	12.41 ± 0.80*	9.36 ± 1.52*

Liver/body weights ± S.D.

Group	males	females
1	3.75 ± 0.17	3.56 ± 0.17
2	3.84 ± 0.19	3.88 ± 0.37
3	4.08 ± 0.20*	4.02 ± 0.24*
4	4.50 ± 0.20*	4.53 ± 0.49*

Liver/brain weights ± S.D.

Group	males	females
1	539.40 ± 60.33	391.30 ± 42.34
2	553.74 ± 71.65	431.46 ± 39.44
3	597.03 ± 54.39*	444.73 ± 47.64*
4	651.04 ± 40.03*	501.31 ± 79.72*

b. Gross pathology

In the pathology summary report the study author stated that "in a few rats of the high dose group and of the solvent control group, small white foci were observed on the lungs. In addition a small number of minor lesions were encountered in various organs as described in the special part of this report". There are no macroscopic summary tables included in this report. This "special part" of the study text could not be found.

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c. Microscopic pathology

1) Non-neoplastic
In the liver of both males and females, according to the study text, there was minimal to slight centrilobular hypertrophy characterized by increased homogeneity of cytoplasm and reduced basophilia in enlarged hepatocytes. Females shoed a high dose effect, while in males, there was no NOEL (see table III). In males, the hepatocellular alteration increased in a dose-related manner.

In lungs, multifocal alveolar disruption with alveolar extensions were noted in all dose groups including controls.

Other microscopic changes seen could not be attributed to treatment.

TABLE III

Centrilobular Hypertrophy

Liver	group 1	2	3	4
Males	0	4	6	10
Females	0	0	0	7

2. Neoplastic: No neoplasms were detected during the study.

Discussion:

Effects were detected in group 4 showing signs of toxicity 6 hours after initial exposure. There was an increase in group 4 female methemoglobin levels, however, the data were not presented to confirm or deny this statement. Total bilirubins were increased in the mid and high dose groups. The study stated that direct bilirubins were also elevated, but did not give the data to confirm this statement. Group 3 and 4 male and female absolute and relative liver weights were increased, and centrilobular hypertrophy of the liver was seen in high dose females and at all doses for males.

NOEL < 100 mg/CBM (LDT) based on centrilobular hypertrophy seen in males at all doses tested.

Core Classification: supplementary

Trifluralin toxicology reviews

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Reviewed by: Marcia van Gemert, Ph.D. 12/4/86
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)
12/4/86

005898

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Toxicity in Mice

TOX. CHEM. NO.: 889

ACCESSION NUMBER: 258997

MRID NO.: ?

TEST MATERIAL: Trifluralin Technical

SYNONYMS: treflan, HOE 38474 OH AT210

STUDY NUMBER(S): 008842

SPONSOR: Hoechst Aktiengesellschaft

TESTING FACILITY: RCC Switzerland

TITLE OF REPORT: 13 Weeks Toxicity Study in Mice

AUTHOR(S): Dr. P. Suter

REPORT ISSUED: June 15, 1983

CONCLUSIONS: There is no NOEL for liver-to-body weight ratios in female mice. Albumin levels were decreased at all dose levels in mice, however, the significance of this is not known. Only limited histopathology was performed in this study. It was basically a rangefinding study for the chronic study.

NOEL < 400 ppm

Classification: core-Supplementary

A. MATERIALS:

1. Test compound: Trifluralin, Description: solid red
Batch #AZ01751, Purity>99%, contaminants: list in CBI appendix

2. Test animals: Species: Mice, Strain: NMRI KFM-Han (outbred)
SPF quality:
Age: 4 weeks,
Weight: males, 19-26 grams, females, 16-20 grams
Kleintierfarmadoerin AG 4414 fuellinsdorf, Switzerland

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 13 weeks male	female
1 Cont.	0	10	10
2 Low (LDT)	400	10	10
3 Mid (MDT)	1000	10	10
4 High(HDT)	2500	10	10

2. Diet preparation

Diet was prepared once at the beginning of the experiment and stored presumably at room temperature. Samples of treated food were analyzed for stability and concentration at the beginning and end of the experiment.

Results - Test substance concentration in diet results are on appendei page 10. The test substance appears stable at up to 1000 ppm for 11 weeks in the diet. However, the 3000 ppm diet at 11 weeks was somewhat lower in 2 of the 3 samples tested.

3. Animals received standard Kliba 343 rat/mouse maintenance diet and water ad libitum.

4. Statistics - The statistical procedures which were utilized in analyzing the numerical data are appended on appended page 1.

5. Quality assurance statement was signed and submitted with the study.

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C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. Neurologic status was also measured.

Mortality (survival)
Only one animal (high dose female) died at day 80 on test due to septicemia caused by a subsequent abcess. No other deaths were reported.

Toxicity:
No clinical signs of toxicity were observed.

Neurologic status:
No treatment-related neurological signs of toxicity were apparent.

2. Body weight

Animals were weighed weekly.

Results: There were no treatment-related changes in body weights.

3. Food consumption and compound intake

Consumption was determined over a 7-day and mean daily diet consumption was calculated and reported weekly. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Food consumption/Food Efficiency/Compound Intake were calculated at weekly intervals based on results of mean food consumption and mean body weights.

Results:
There were no treatment-related changes in food consumption or food efficiency.

4. Ophthalmological and hearing examinations

Performed prior to and at the end of the treatment on all animals.
Hearing tests were performed prior to and at the end of the treatment period (10kHz, 80Db, 30 msec, 5 tests with 2 second pauses between tests. Preyers reflex = positive reaction.)

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Results:
No treatment-related effects were seen in the ophthalmological and hearing exams.

5. Blood was collected before treatment and at 13 weeks for hematology and clinical analysis from 10/sex/group. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood Clotting Measurements	X	Methemoglobin
	(Thromboplastin time)	X	Heinz bodies
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Hematology Results:

There was an increase in Heinz body formation in both males and females at the high dose. The study text states that this suggests an increased rate of oxidation of hemoglobin to toxic hemolytic anemia. They state that the percentage of erythrocytes containing Heinz bodies averaged between 41 to 75%. The size of the Heinz bodies averaged between 2 and 4 microns in diameter. Most were stated to occur as a single entity within a single red cell and tended to lie close to the periphery. See appended pages 2 and 4.

Related to this finding there also occurred a statistically significant ($p < 0.05$ for males and 0.01 for females) increase in methemoglobin at the high dose for both males and females and at the middle dose also. See appended pages 2 and 4 for details.

At the high dose in males there was a significant ($p < 0.01$) increase in platelet count. See appended page 2.

In the middle and high dose males there was a significant ($p < 0.01$) decrease in monocytes. (See appended page 3) This however, did not occur in females. (see appended page 5 for female data)

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5. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	gamma glutamyl transferase		
X	glutamate dehydrogenase		

* Required for subchronic and chronic studies
° Not required for subchronic studies

Results:

1. Creatinine levels were significantly increased in both males and females at the high dose ($p < 0.05$ for males and 0.01 for females). Females at the middle dose also had increased creatinine levels ($p < 0.05$). See appended pages 6 and 8.

2. Urea levels were increased in both males and females at the high dose ($p < 0.01$) but this was not present in females. See appended pages 6 and 8.

3. Sodium levels were increased in both males and females at the high dose ($p < 0.05$ for males and 0.01 for females). Females at the middle dose also had increased sodium levels ($p < 0.01$) See appended pages 6 and 8 for details.

4. Chloride levels were increased in males only in the middle dose with a $p < 0.01$. However, they were increased in the females at the high dose ($p < 0.05$). see appended pages 7 and 9 for exact numbers.

5. In females at the high dose both total bilirubin and cholesterol levels were increased significantly ($p < 0.01$). However, levels remained within controls for males. See appended page 8.

6. Albumin was decreased at all dose levels in females ($p < 0.01$). There did not appear to be a dose-response relationsnip. The biological significance is not clear. See appended page 9.

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6. Urinalysis was not performed.

7. Sacrifice and Pathology -
All animals that died and that were anaesthetized by intraperitoneal injection of pentabarbitol and killed by exanguination on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination but not necessarily examined. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	.Aorta*	XX	.Brain*†
X	.Salivary glands*	XX	.Heart*	X	Periph. nerve*#
X	.Esophagus*	X	.Bone marrow*	X	Spinal cord (3 levels)*#
X	.Stomach*	X	.Lymph nodes*	X	.Pituitary*
X	.Duodenum*	XX	.Spleen*	X	Eyes with contiguous
X	.Jejunum*	X	.Thymus*		Glandular harderian glands
	.Ileum*		Urogenital	X	.Adrenals*
X	.Cecum*	XX	.Kidneys*†		Lacrimal gland#
X	.Colon*	X	.Urinary bladder*	X	Mammary gland*#
	.Rectum*	XX	.Testes*†		.Parathyroids*††
XX	.Liver*†	X	Epididymides	X	.Thyroids*††
X	Gall bladder*#	X	Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*#
	Respiratory	XX	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	X	.Uterus*	X	Skin*#
X	.Lung* with mainstem bronchi			X	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

The above tissues were fixed for histological exam. However, only the following organs were actually sectioned and examined microscopically. (number of sections per organ/tissue)

Gross lesions/tissue masses
Brain (2)
Heart (1)
Liver (3)
Kidneys (2)
Spleen (1)
Adrenal glands (2)
Testes/ovaries (2)
Bone Marrow (1)

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a. Organ weight

Liver appeared to be the target organ with increases in absolute and relative liver weight perameters, mostly in the mid and top dose animals.
See table I for details.

Table I

Liver-male		Dose- ppm 0	400	1000	2500
Absolute wts.	Mean	1.83	1.86	2.03	2.52**
	S.D.	0.28	0.43	0.41	0.30
liver/body wt ratio	Mean	4.582	4.713	4.988*	5.990**
	S.D.	0.368	0.536	0.493	0.343
Liver/brain wt ratio	Mean	393.035	396.911	422.590	529.771**
	S.D.	69.739	110.294	81.263	66.010
Liver-females					
Absolute wts	Mean	1.43	1.45	1.58	1.92**
	S.D.	0.18	0.17	0.22	0.23
Liver/body wt. ratio	Mean	4.749	5.294*	5.316*	6.878**
	S.D.	0.490	0.306	0.479	0.526
Liver/brain wt. ratio	Mean	297.719	291.040	328.443	400.769**
	S.D.	36.198	31.278	51.816	49.643

* = $p < 0.05$
** = $p < 0.01$

b. Gross pathology

One high dose female died on day 80 on test and two low dose mice killed at termination had subcutaneous nodules seen in the cervical and head region. No other dose-related gross changes were observed.

c. Microscopic pathology

Liver: there was minimal to moderate centrilobular hepatocellular hypertrophy which was dose-dependent. It was noted in 19 high dose (10 male and 8 female), 10 mid-dose (7 males and 3 females) and 2 low dose males. These are considered to be indicative of induction of liver metabolizing enzymes. The nodules seen in gross pathology wre subcutaneous granulomas and absesses which are considered common in this strain of mouse.

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DISCUSSION:

This is a range-finding study done in order to select the proper doses for the chronic study. There was no no-effect level seen for liver-to body weight ratios in the female mice. Albumin levels were decreased at all dose levels. Only very limited histopathology was performed on the tissues saved at sacrifice.

Therefore there was no NOEL determined for this study, and the core classification is supplementary.

Trifluralin toxicology reviews

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Pages 103 through 112 are not included in this copy.

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005898

Reviewed by: Stephanie P. April, Ph.D. Stephanie April delivered 2pm 12/16/86
Section III, Toxicology Branch (TS-769C)
Secondary Reviewer: Marcia van Gemert, Ph.D.
Section III, Toxicology Branch (TS-769C) M. van Gemert 4/22/86

DATA EVALUATION REPORT

Study Type: Subchronic Feeding Study in Rats

Accession Number: 258998

Test Material: Trifluralin

Synonyms: HOE 38474

Study Number: 0620

Testing Facility: Pharma Forshung Toxikologia
Hoechst AG, P.O. Box 800320
6230 Frankfurt (M) 80

Authors: Dr. Schutz, Dr. Weigand, and Prof. Kramer

Report Issued: November 18, 1980

Conclusion:

There are treatment-related trends and effects in several parameters including female liver/body weight increases and pituitary to body weight decreases with no NOEL. The requested information which is detailed in the discussion is necessary for the reviewer prior to making any further conclusions.

Classification: Core Minimum

A. Materials:

1. Test Materials: HOE 38474-technical active ingredient, Code: HOE 38474 OH AT 204; Common name: Trifluralin; Certificate of Analysis No. 01078; Dated August 15, 1979. α,α,α,-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.

2. Test Animals: Four-week-old male (92 g) and female (90 g) Hoe: Wistar (SPF71) rats from Hoechst AG, animal breeding station Kastengrund were used.

B. Study Design:

1. The animals were randomly assigned to the following 4 groups of 20 males and 20 females:

Table 1

	Test Group	Dose (ppm)	Males	(Animal Nos.)	# Females	(Animal Nos.)
a.	Control	0	20	(00001-00020)	20	(00021-00040)
b.	Low (LDT)	800	20	(00041-00060)	20	(00061-00080)
c.	Mid (MDT)	2000	20	(00081-00100)	20	(00101-00120)
d.	High (HDT)	5000	20	(00121-00140)	20	(00141-00160)

The dosages were based upon oral LD_{50} studies in the reporting lab of 1.93 g/kg for the males and 2.27 mg/kg for the females and by the Hoechst Company.

The trifluralin was administered daily for 3 months and one half of the animals (10 per sex per group) were observed in a followup period for 15 days without treatment.

2. Diet Preparation - The diet was prepared as premixture of Altromin-1321 with trifluralin. The source of the diet was not given nor was the temperature or method of storage. The treated diet was prepared fresh weekly and samples were analyzed for stability and concentration at Analytical Laboratory (Bereich Co., Pfl. Analyse) of Hoechst AG.

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

a. Simultaneous Comparison According to Nemenyi;
b. Simultaneous Comparison According to Tukey; and
c. 3-class Contingency Table.

The following parameters were tested for a significance level of $p = 0.05$: body weight, leucocytes, coagulation time, thrombocytes, haemoglobin, erythrocytes, haematocrit, reticulocytes, sodium, potassium, inorganic phosphorus, uric acid, bilirubin, creatinine, glucose, urea-N, calcium, chloride, SGOT, SGPT, a.P. and all relative organ weights.

5. Quality Assurance was reported to the Management of the Research Department and the Study director at regular intervals: 9/14/79, 10/9/79, 11/26/80 by Harston

assuring inspection of the experiment.

C. Methods and Results:

1. Observations - The animals were inspected daily for signs of toxicity, mortality, and behavior. The teeth visible mucosa and neurological status were checked monthly.

   Results - No deaths or moribund observations occurred throughout the 3-month study. There was no difference in behavior in the treated animals as compared to the controls and no signs of toxicity were observed. There were no results given on the observations of the teeth, visible mucosa or neurological status.

2. Body Weight - The animals were weighed weekly throughout the experiment.

   Results - Only in the high dose group (HDT) and after day 8 were there reduced weight gains. These statistically significant differences were more pronounced in the males than in the females and were probably treatment related.there were no statistically significant differences in the changes from control in the low and mid-dose groups.

   Animals retained untreated after termination of study reversed this effect.

Table 2. Body Weight (g) (% of Control Weight)
Body Weight Gain (g)

Dose (ppm)/sex	DAY 1	DAY 92
0/M	148.3	404.2/255.8
800/M	147.2 (99.29)	394.9 (97.6) / 247.7
2000/M	146.6 (98.85)	399.2 (98.7) / 252.6
5000/M	143.0 (96.4)	376.0 (93.0) / 233.0*
0/F	129.4	221.3/91.9
800/F	125.5 (96.98)	222.9 (100.7) / 97.3
2000/F	126.1 (97.4)	216.4 (97.17) / 90.3
5000/F	127.0 (98.1)	210.5 (95.1) / 83.5*

* = P <0.05

3. Food Consumption and Compound Intake - The daily food consumption was calculated from weekly checks. The compound intake was calculated from this and the body weight data.

Results - With exception of the first treatment week of the high dose group (adapation to the palatability of high concentrations of trifluralin) all groups consumed the same amount of food.

Figures 2 and 3 were photocopied to demonstrate the similarity in food consumption in the groups and are labeled Figures 1 and 2 in this document.

Food efficiency was not calculated or referred to in the report; however, Table 3 gives the food consumption in grams and as a percent body weight which is adequate information.

Table 3. Food Consumption Per Day

Dose	Day 8	% Body Weight	Day 92	% Body Weight
Male				
0	19.87 g	11.64	21.57 g	5.375
800	18.7 g	11.11	21.63 g	5.5
2000	18.9 g	11.35	22.51 g	5.69
5000	16.66 g	10.48	21.83 g	5.67
Female				
0	17.02 g	12.08	15.71 g	7.102
800	15.46 g	11.33	15.62 g	7.034
2000	15.41 g	11.45	14.64 g	6.81
5000	13.88 g	10.38	14.08 g	6.79

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Summary results of compound consumption mg per kg body weight per day throughout the 3 months was given as:

Table 4. Compound Consumption

Dose ppm (mg/kg)	Sex	Actual Mean Trifluralin Consumption (mg/kg/day)	Day 8	Day 50	Day 92
0 (0)	M	0	0	0	0
	F	0	0	0	0
800 (80)	M	59	88.9	53.75	44.11
	F	69	90.71	63.32	56.275
2000 (200)	M	154	227.17	137.048	113.824
	F	168	228.92	155.628	136.201
5000 (500)	M	392	524.195	363.25	283.891
	F	421	519.084	405.811	339.508

4. Ophthalmological examinations were performed monthly on all animals to check for cloudiness and pathological changes.

   Results - There were no pathological changes throughout the test period.

5. Blood was collected before treatment and at 7, 13, and 16 weeks of the study for hematology and clinical analysis from 10 male and 10 female nonfasted rats per group taken from a sublingual vein. The parameters examined were hemoglobin, erythrocytes, hematocrit, reticulocytes, Heinz bodies, thrombocytes, coagulation time, leukocytes, and differential blood count. Reticulocytes and Heinz bodies were only determined from the last blood samples taken. Data processing techniques were used to determine the median cell volume, median corpuscular haemoglobin content, and median corpuscular hemoglobin concentrations.

   Results - Measurements of most of these parameters yielded values which were not significantly different from control values.

   The following table indicates that there were random significant differences from control in some of the values of erythrocytes, leukocytes, hemoglobin and coagulation time.

   As can be seen from these significant differences there was a dose-related significant trend in decrease in hemoglobin at the high dose while there was only a significant decrease in the intermediate-dose females at the 7-week interval. There were nonsignificant dose-related trends in hemoglobin in both males and females. There were also dose-related decreases in coagulation time in the male.

Table 5. Significant Changes from Hematological Values of the Control Group

Parameter		Leukocytes x10^9/L			Coagulation Time (sec.)			Hemoglobin (g/L)			Erythrocytes (x10^{12}/L)		
Time		1	2	3	1	2	3	1	2	3	1	2	3
	Dose												
Male	0	9.98	11.54	7.38	105.8	105.2	113.5	124.3	158.6	148.5	5.998	8.154	7.784
	800	7.16 −	10.04	6.86	89.7 −	107.3	90.0	125.0	154.1	141.4	5.936	8.035	7.591
	2000	7.74 −	10.08	8.27	93.8	110.9	86.6 −	126.5	154.1	147.7	5.761	8.027	7.884
	5000	8.44	8.84 −	10.66	93.4	104.2	92.6	126.4	149.8 −	135.2 −	5.903	7.770	7.162
Female	0	7.61	7.22	6.33	89.4	120.1	86.7	124.7	140.7	142.6	5.841	7.105	7.437
	800	7.12	6.87	6.26	84.5	129.5	98.3	127.9	131.0	151.2	5.979	6.568 −	7.888 +
	2000	7.03	7.14	7.47	85.3	147.7	89.3	128.9	130.5 −	139.4	6.281	6.630	7.482
	5000	7.01	6.49	7.08	84.8	150.8 +	90.6	129.0	132.6	134.0	6.034	6.761	6.978 −

+ = significant increase from control
p = < 0.05
− = significant decrease from control
p = < 0.05
1 = initial
2 = intermediate - 7 weeks
3 = final - 13 weeks

6. Clinical Chemistry - Ten nonfasted rats per sex per group had serum analyses at the beginning of study, and at week 7, 14, and 16 for the following parameters: glucose, urea-N, SGOT, SGPT and alkaline phosphatase. Final blood samples from exsanguination were used in addition for determination of sodium, potassium, inorganic phosphorus, uric acid, bilirubin, creatinine, calcium, chloride, and methemoglobin.

The following chemicals were not measured: magnesium, cholinesterase, creatinine phosphokinase, lactic acid dehydrogenase, albumin, cholesterol, globulins, total proteins, and triglycerides.

Results - The parameters affected with significant increases or decreases were: SGPT, AP, inorganic phosphorus, uric acid. In the low and mid dose there is a lack of trends and the registrant states that the values which significantly deviated from control are within the range for this strain of rats in their laboratory. The decreased SGPT (39.7 to 31.7 u/L) and AP (215.7 to 152.0 U/L) in the male seem to be related to treatment as compared with the control at the final day of dosing.

Table 5a. Clinical Chemistry

		Day							
		1		44		93			
Parameter		SGPT U/L	AP U/L	SGPT U/L	AP U/L	SGPT U/L	Inorganic Phosp.	Uric Acid uMol/L	AP U/L
Sex	Dose								
M	0	48.2	376.8	36.5	337.2	39.7	2.005	92.2	215.7
F	0	41.8	466.3	32.9	262.3	34.3	2.016	159.8	109.1
M	800	45.1	454.8^{+}	33.5	324.0	38.6	2.356	103.8	201.7
F	800	38.2	439.5	28.8	208.5^{-}	36.3	2.433^{+}	98.8^{-}	129.4
M	2000	40.5	396.8	30.6	305.1	37.6	2.705^{+}	159.2	195.3
F	2000	34.9	467.8	28.3	176.3^{-}	36.4	2.544^{+}	99.4^{-}	139.2
M	5000	37.2-	466.3	27.1-	248.6^{-}	31.7-	2.829^{+}	104.6	152.0^{-}
F	5000	40.9	474.1	29.6	187.8^{-}	33.7	2.607^{+}	101.4^{-}	115.3

Of the parameters given in Table 5a (those with statistically significant differences from control) only SGPT and AP were measured on Day 1 and Day 44. On Day 1 the high dose males had a significant decrease in SGPT ($P<0.05$ from control while the low dose males had a significant increase of AP from control ($P<0.05$).

At the intermediate time (Day 44) the males had significant decreases in SGPT and AP at the high dose and the females had significant decreases from control in AP with no NOEL. At 93 days the males had a significant decrease in SGPT and AP and an increase in Inorganic Phosphorus at the mid and high dose level. The females at this time had no NOEL for either a decrease in uric acid or an increase in inorganic phosphorus.

7. Urinalysis - The overnight urine of 10 rats per sex per group prior to the the experiment, at week 7 and 13 was tested for determination of appearance, color protein, hemoglobin, glucose, bilirubin, pH value and sediment. At week 16 after recovery only appearance and color were recorded. The volume, specific gravity, ketones, bilirubin, nitrates and urobilinogen were not determined.

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Results - There was a dose dependent color intensity of dark yellow to reddish brown in the urine indicating absorption. At the mid and high-dose level the coloration disappeared from the urine by 2 days posttreatment.

8. Sacrifice and Pathology - All animals that were sacrificed on schedule* were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

*Half of the animals (10 per sex per group) were sacrificed 24 hours after discontinuation of treatment and the other half were sacrificed 15 days after termination of treatment.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Salivary glands*	X	Aorta	XX	Brain
X	Esophagus	XX	Heart	XX	Pituitary
X	Stomach	X	Bone marrow	X	Eyes (optic n.)
X	Duodenum*	X	Lymph nodes		Glandular
X	Jejunum	XX	Spleen	XX	Adrenals
X	Ileum	X	Thymus	X	Mammary gland*
X	Cecum		Urogenital	XX	Thyroids
X	Colon	XX	Kidneys		Other
X	Rectum	X	Urinary bladder	X	Bone
XX	Liver	XX	Testes	X	Skeletal muscle
X	Pancreas	X	Epididymides	X	Skin
	Respiratory	X	Prostate	X	All gross lesions
X	Trachea	XX	Seminal vesicle	X	and masses
XX	Lung	XX	Ovaries	X	Fatty tissue from abdominal cavity of mid and high dose
X	Diaphragm	X	Uterus		

The tongue, peripheral nerve, spinal cord (3 levels), adrenals, lacrimal gland, and parathyroids, were not saved. The above marked tissues were fixed and examined histologically. The adrenals, peripheral nerve, and lacrimal gland were listed in the Pesticide Assessment Guidelines of 1982.

Gross Pathology - There were no significant findings at autopsy. Slight increase in fat cells with no morphological changes to fat cells in the high dose groups in the bone marrow and lymphatic hyperplasia of the intestine were seen.

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The report states that in the high dose animals:

> The following organs were not examined: aorta of Nos. 123, 126, 129, 151 and 156; optic nerve of Nos. 126, 127, 133, 135, 136, 139, 140, 142, 143, 146, 147, 152, 155, 156 and 157; pituitary glands of Nos. 126, 134, 138, 146, 150 and 156; bone marrow of Nos. 121, 131, 143, 144, 149, 150, 151, 153 and 160; hilar lymph nodes of Nos. 126, 130 and 134; iliac lymph nodes of Nos. 123, 126, 130, 132, 137, 138, 152, 153, 157, 158 and 159; endometrium of Nos. 144 and 147; thyroid of No. 145; uterus of Nos. 158, 160 and mamma [probably refers to mammary gland] of animal No. 141.

There were no trifluralin-induced morphological changes observed.

Results:

a. Organ Weight - Of the organs that were weighed the liver had a dose dependent increase in absolute weight as can be seen in Table 6 in both males at the mid and high dose and in females with no NOEL. The reviewer calculated the significance with a program for calculating p-values with Student's T-test created for the Lextitron (obtained from D. Ritter and verified by a software package from the statistics section on an IBM PC).

   There were dose-dependent changes, significant at all dose levels, in organ/body weights for pituitary and liver of females with no no-effect level. This is seen as a decrease in the case of the pituitary and an increase in the case of the liver. In the male there was a dose-related increase in the relative liver weights which was significant only at the intermediate and high dose.

b. Microscopic Pathology - The report states that the tissues of all experimental animals were examined microscopically. Table 8 gives the findings mentioned in the report - note the discrepancies between the numbers given in the report in two places (i.e., total numbers which were given in the report for kidney pyelectasis were 13, 8, 3, and 17 (as given on page 398 of the report) and the number of animal numbers (as given on pages 400, 402, 404, and 406 of the report). The correct total numbers are shown in Table 8.

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Table 6. Absolute Organ Weights (excerpted from report)

a) Male

FINAL VALUE 1 DAY AFTER LAST DOSE

M 1 = Control MALE M 2 = 800 PPM MALE
M 3 = 2000 PPM MALE M 4 = 5000 PPM MALE

	N	BODY WEIGHT	HEART	LUNGS	LIVER	KIDNEYS	SPLEEN	TESTES	ADRE-NALS	THYROID	BRAIN	PITU-ITARY	SEMINAL VESICLE
M 1 MEAN	10	386.3	1.25	2.16	12.51	2.468	0.720	3.093	0.061	0.023	1.959	0.011	1.979
SD		51.9	0.12	0.85	3.22	0.253	0.097	0.791	0.007	0.003	0.103	0.004	0.579
N		10	10	10	10	10	10	10	10	10	10	10	10
M 2 MEAN	10	388.3	1.20	1.80	14.33	2.439	0.754	3.502	0.058	0.023	1.912	0.010	1.964
SD		28.7	0.13	0.24	1.68	0.191	0.128	0.242	0.007	0.007	0.123	0.001	0.413
N		10	10	10	10	10	10	10	10	10	10	10	10
M 3 MEAN	10	395.5	1.37	1.61	16.14*	2.517	0.768	3.335	0.063	0.022	1.923	0.010	1.808
SD		29.0	0.41	0.39	2.17	0.164	0.153	0.485	0.004	0.004	0.124	0.003	0.553
N		10	10	10	10	10	10	10	10	10	10	10	10
M 4 MEAN	10	362.8	1.19	1.70	16.98**	2.327	0.840	3.427	0.066	0.022	1.974	0.009	2.085
SD		40.2	0.11	0.24	1.89	0.320	0.228	0.236	0.014	0.004	0.137	0.002	0.336
N		10	10	10	10	10	10	10	9	10	10	10	10

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 6 (Cont'd)

a) Female

FINAL VALUE 1 DAY AFTER LAST DOSE

F 1 = Control FEMALE
F 2 = 800 PPM FEMALE
F 3 = 2000 PPM FEMALE
F 4 = 5000 PPM FEMALE

	N	BODY WEIGHT	HEART	LUNGS	LIVER	KIDNEYS	SPLEEN	OVARIES	ADRE-NALS	THYROID	BRAIN	PITU-ITARY
F 1 MEAN	10	224.5	0.84	1.13	7.64	1.502	0.501	0.118	0.075	0.020	1.770	0.014
SD		21.8	0.04	0.17	0.74	0.147	0.092	0.028	0.010	0.008	0.059	0.004
N		10	10	10	10	10	10	10	10	10	10	10
F 2 MEAN	10	220.5	0.84	1.89	8.50*	1.453	0.641	0.133	0.073	0.021	1.840	0.010
SD		13.9	0.07	0.61	0.82	1.170	0.148	0.028	0.011	0.005	0.073	0.002
N		10	10	10	10	10	10	10	10	10	10	10
F 3 MEAN	10	220.2	0.84	1.54	8.51*	1.466	0.583	0.127	0.063	0.019	1.793	0.009
SD		21.9	0.09	0.41	0.99	0.196	0.122	0.014	0.005	0.005	0.101	0.001
N		10	10	10	10	10	10	10	10	10	10	10
F 4 MEAN	10	209.4	0.83	1.44	10.58***	1.470	0.563	0.129	0.067	0.023	1.776	0.010
SD		13.4	0.07	0.49	1.08	.166	0.102	0.031	0.013	0.006	0.098	0.001
N		10	10	10	10	10	10	10	10	10	10	10

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 7. Relative Organ Weights (% body weight) (excerpted from report)

a) Male

FINAL VALUE 1 DAY AFTER LAST DOSE

M 1 = Control MALE M 2 = 800 PPM MALE
M 3 = 2000 PPM MALE M 4 = 5000 PPM MALE

	N	BODY WEIGHT	HEART	LUNGS	LIVER	KIDNEYS	SPLEEN	TESTES	ADRE-NALS	THYROID	BRAIN	PITU-ITARY	SEMINAL VESICLE
M 1 MEAN	10	386.3	0.33	0.56	3.17	0.646	0.190	0.787	0.016	0.0060	0.518	0.0027	0.5008
SD		51.9	0.06	0.19	0.62	0.076	0.03[illegible]	0.151	0.002	0.0008	0.093	0.0009	0.1215
N		10	10	10	10	10	[illegible]0	10	10	10	10	10	10
M 2 MEAN	10	388.3	0.31	0.47	3.69	0.629	0.194	0.907	0.015	0.0059	0.495	0.0026	0.5051
SD		28.7	0.02	0.06	0.27	0.027	0.027	0.102	0.003	0.0017	0.050	0.0005	0.0944
N		10	10	10	10	10	10	10	10	10	10	10	10
M 3 MEAN	10	395.5	0.35	0.41**	4.07*	0.638	0.194	0.843	0.016	0.0056	0.489	0.0026	0.4622
SD		29.0	0.10	0.10	0.32	0.038	0.030	0.109	0.003	0.0011	0.054	0.0007	0.1537
N		10	10	10	10	10	10	10	10	10	10	10	10
M 4 MEAN	10	362.8	0.33	0.47	4.69**	0.641	0.230	0.954	0.019	0.0061	0.549	0.0026	0.5790
SD		40.2	0.02	0.08	0.30	0.046	0.042	0.111	0.004	0.0015	0.061	0.0006	0.0999
N		10	10	10	10	10	10	10	9	10	10	10	10

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table 7 (Cont'd)

b) Female

FINAL VALUE 1 DAY AFTER LAST DOSE

F 1 = Control FEMALE F 2 = 800 PPM FEMALE
F 3 = 2000 PPM FEMALE F 4 = 5000 PPM FEMALE

	N	BODY WEIGHT	HEART	LUNGS	LIVER	KIDNEYS	SPLEEN	OVARIES	ADRE-NALS	THYROID	BRAIN	PITU-ITARY	
F 1 MEAN	10	224.5	0.38	0.51	3.41	0.669	0.224	0.054	0.034	0.0090	0.796	0.0064	
SD		21.8	0.04	0.09	0.19	0.024	0.041	0.016	0.005	0.0038	0.096	0.0020	
N		10	10	10	10	10	10	10	10	10	10	10	
F 2 MEAN	10	220.5	0.38	0.86*	3.85**	0.659	0.292*	0.060	0.033	0.0093	0.839	0.0047-	*
SD		13.9	0.03	0.26	0.27	0.068	0.070	0.012	0.004	0.0030	0.077	0.0011	
N		10	10	10	10	10	10	10	10	10	10	10	
F 3 MEAN	10	220.2	0.38	0.70	3.86**	0.664	0.263	0.058	0.029	0.0088	0.822	0.0043-	**
SD		21.9	0.02	0.17	0.18	0.032	0.034	0.006	0.003	0.0028	0.097	0.0005	
N		10	10	10	10	10	10	10	10	10	10	10	
F 4 MEAN	10	209.4	0.40	0.69	5.05***	0.702	0.269	0.062	0.032	0.0111	0.850	0.0049-	*
SD		13.4	0.03	0.21	0.36	0.060	0.046	0.015	0.006	0.0028	0.047	0.0012	
N		10	10	10	10	10	10	10	10	10	10	10	

* p< 0.05

** p< 0.01

*** p< 0.001

Table 8. Number of Lesions (Individual Animal Numbers)

Dose	Page	No. Kidney pyelectasis	Ovarian cysts
0	pg.400	13 (1, 2, 8, 9, 11, 12, 13, 14, 16, 20, 27, 30, 35)	-
800	pg 402	7 (44, 48, 58, 67, 68, 71, 79)	9 (61, 63, 65, 66, 69, 70, 73, 78, 79)
2000	pg. 407	12 (82, 88, 89, 94, 95, 97, 101, 104, 109, 114, 117, 119)	1 (116)
5000	pg. 406	17 (121, 124, 126, 127, 129, 130, 132, 133, 135, 138, 140, 153, 154, 155, 157, 158, 159)	4 (145, 146, 155, 160)

Other changes in all groups included: megakaryocytosis of fat cells in bone marrow; lymphatic hyperplasia in intestines, unrounded shape of islets or pyknetic islet cells in pancreas and orchiatropy in one animal. These tissues were selected for frequency of lesions.

Discussion:

Since statistically significant deviations from control for the clinical chemistry values were obtained in this study, the company should supply EPA with historical values with data for 90 days for this strain in their laboratory so that more accurate assessments can be made by the reviewer. Historical data for organ weights and organ to body weight ratios for control animals of this same strain at 0 time and 3 months in this laboratory is requested.

There were discrepancies in the number of microsccpic lesions given in one part of the text and the number of individual animal numbers given in another part of the text. (This is specified in Table 8 and the paragraph above it.)

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Tox Chem No. Trifluralin | File Last Updated _____ | Current Date 12/16/86

Study/Lab/Study #/Date	Material	EPA Accession No.	Results	TOX Category	CORE Grade
Subchronic feeding in rats	Technical	258998	Female liver/body weight absolute liver weight and pituitary/body weight were different from control with No NOEL; The male absolute Liver-Weight and liver/body weight at the mid and high dose levels were significantly different from control.	—	Core Minimum

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-02-4225
DYNAMAC No. 208-C
August 8, 1986

005898

DATA EVALUATION RECORD

TRIFLURALIN

Six-Month Subchronic Toxicity Study in Dogs

STUDY IDENTIFICATION: Brunk, Weigand, and Kramer. Toxicological testing of trifluralin (Hoe 38474 OH AT 204) by repeated oral administration to beagle dogs for six months. (Unpublished study No. 633 by Pharma Forschung Toxikologie for Hoechst Aktiengesellschaft, Frankfurt, West Germany.) Accession Nos. 258999-259000.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 8-8-86

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1. CHEMICAL: Trifluralin; α,α,α-trifluoro2,6-dinitro-N,N-dipropyl-p-toluidine.

2. TEST MATERIAL: Trifluralin (Hoe 38474 OH AT 204) production standard, lot No. 8900, batch 1/79, had a stated purity of 96.1%.

3. STUDY/ACTION TYPE: Six-month subchronic toxicity study in dogs.

4. STUDY IDENTIFICATION: Brunk, Weigand, and Kramer. Toxicological testing of trifluralin (Hoe 38474 OH AT 204) by repeated oral administration to beagle dogs for six months. (Unpublished study No. 633 by Pharma Forschung Toxikologie for Hoechst Aktiengesellschaft, Frankfurt, West Germany.) Accession Nos. 258999-259000.

5. REVIEWED BY:

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Date: 08-07-1986

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Date: 8-7-86

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of the study, trifluralin was toxic to male and female dogs when fed for 6 months at levels of 400, 1000, or 2500 ppm in the diet. The following compound-related effects were observed at all dose levels: enlarged livers, discolored kidneys, corneal vascularization, hemolytic anemia, and increased serum alkaline phosphatase activity. An exceeded maximum tolerated dose (MTD) and aversion to food caused starvation in two high-dose animals (one male, one female) and resulted in their moribund sacrifice after 86 and 39 days of dosing, respectively. Reductions in body weight gain and food consumption were observed in the high-dose animals. There were no distinct or consistent histologic alterations that could be related to the increased liver weights and discoloration of kidneys. In some high-dose males, testes were smaller than normal.

The NOEL for systemic toxicity was not achieved; the LOEL is 400 ppm, the lowest dose tested.

B. Core Classification: Core Supplementary.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A Materials and Methods. (See Appendix A for details.)

1. A single lot (No. 8900) of trifluralin was used to prepare the test diets throughout the study. The test compound was 96.1% pure. The required amounts of the test compound were premixed with cornmeal, and the mixture was blended into ready-made dog food, 'Vipromix' from NAGUT Kraftfuterwerke (Dr. Müller), to achieve 0-, 400-, 1000-, and 2500-ppm levels of concentration in the test diets. Fresh batches of test diets were prepared each day throughout the study.

2. Approximately 9-month-old purebred English beagle dogs of the Hoe: BEAK strain (Hoechst breed) were used in this study. Four groups of six dogs/sex with initial mean body weights of 12.4 kg (9.7-14.3) for males and 11.6 kg (10.3-12.5) for females were fed the test diets for 6 months. Following the regular period (6 months) of feeding, two animals from each dietary group were left on the control diet for a further period of 4 weeks for recovery.

---

[1] Only items appropriate to this DER have been included.

Animals were housed in individual kennels in a room maintained at 18°C. Dogs were fed in individual daily portions of 1 kg for the males and 0.8 kg for the females.

3. Body weights were determined at the start of the study and weekly thereafter. The portions of the leftover food were recorded each morning to determine food consumption.

4. Animals were examined daily for general health, behavior, and mortality. Neurologic, ophthalmic, ear, and dental examinations were conducted prior to initiation of the study, after about 6 and 12 weeks, and at the end of the study or during the recovery period.

5. Hematologic and clinical chemistry parameters and coagulation times were determined pretest, every 4 weeks thereafter, and before the termination of study. Additional determinations were made on eight animals at the end of recovery period. Urine samples were analyzed before the start of the study, after 6 weeks and 3 months, and at the end of dosing or during the recovery period.

6. Hepatic and renal functions were tested prior to initiation, after 3 months, and at the end of dosing or during the recovery period.

7. All survivors of the scheduled 6 month sacrifice, animals sacrificed after recovery, and two animals sacrificed during the study received complete postmortem examinations. All macroscopic abnormalities were recorded. A total of 14 organs were weighed. Animals were sacrificed by intravenous injections of the killing drug T61-HOECHS and exsanguinated by opening the carotid artery.

8. A complete microscopic examination of representative samples of protocol-designated tissues was made. A minimum of 40 tissues/organs were examined.

9. Statistical methods were not described in the text. Methods used were footnoted in the tables of the report.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Dietary Analysis: No dietary analyses were given in the report.

B. Clinical Observations and Mortality: One 2500-ppm female showed a continuous compound-related decline in general health conditions after 4 weeks and three 2500-ppm males showed similar effects about midway through the study, respectively. The high-dose

female (No. 1228) rejected food, exhibited mild neurologic impairment, and was sacrificed after 39 days of dosing. One high-dose male (No. 1204) exhibiting cachexia due to a marked reduction in food intake over a longer period was sacrificed after 85 days of dosing. No spontaneous deaths occurred during the study.

In the early part of the study, traumatic corneal lesions (pale gray-whitish spots or small radiating foci of opacity) were noted in 3 of 12 control dogs, 2 of 12 receiving 400 ppm, and 2 of 12 receiving 2500 ppm. These lesions were considered to be caused by introduction of sand into the dogs' eyes while digging and scraping in the run area. However, by the end of the study there was a dose-related increase in severity of the corneal lesions (necrosis and ulceration) and a dose-related increase in vascularization of the damaged areas. Table 1 summarizes data on ophthalmic lesions. The lesions persisted in recovery animals.

TABLE 1. Summary of Ophthalmic Lesions During 6 Months of Trifluralin Feeding[a]

Observation	Dose Level (ppm)			
	0	400	1000	2500
Corneal lesions	7/12[b]	7/12	8/12	9/10
Corneal vascularization	0/12	3/12	5/12	7/12

[a]Summary table No. 2, page 20 of the report.

[b]Second number is the number of male and female dogs examined.

At study termination, the visible mucosae of one male receiving 1000 ppm (No. 1193) looked pale. In the second half of the study, the visible mucosae in the high-dose dogs were pale and in those killed right after termination of dosing at 6 months were discolored (yellowish).

C. Body Weights: Mean body weights were presented graphically. Neither the weekly nor mean weekly individual body weight values were presented. Individual weight data were present only at initiation and termination. Table 2 summarizes mean weights and weight gains by sex and group. It was noted from the graphs presented in the report that mean weights in high-dose males and females were lower than controls as early as weeks 4-5 of the study. The high-dose male that was sacrificed moribund at day 86 lost 3.5 kg, and the female sacrificed moribund at day 39 lost

TABLE 2. Group Mean Body Weights (kg ± SD) of Dogs Fed Different Levels of Trifluralin for 6 Months[a]

Dose Level	Initial Weight	Terminal Weight	Weight Gain or Loss (kg ± SD)
		MALES	
Control	11.8±1.49	13.4±1.35	+1.6±0.36
400	12.8±1.61	13.5±1.57	+0.8±0.27
1000	12.5±0.48	13.1±0.37	+0.6±0.74
2500	12.6±1.11	12.0±2.25	-0.5±1.71
		FEMALES	
Control	11.7±0.81	12.7±0.56	+1.0±0.87
400	11.7±0.90	12.5±0.83	+0.8±0.20
1000	11.2±0.71	12.5±0.63	+1.3±0.67
2500	11.7±0.61	11.2±0.98*	-0.5±0.69

[a]Based on six dogs/group except for the 2500-ppm group where only five dogs/group were weighed.

*Significantly different from control by ANOVA followed by Duncan's test for multiple comparisons ($p \leq 0.05$); statistical analyses performed by our reviewers.

2.9 kg body weight. During the 4-week recovery period, one of the two male dogs that had received 2500 ppm gained 0.5 kg and one female from the same group gained 0.9 kg. Weights of other dogs did not change markedly during the 4-week recovery period.

D. Food Consumption: The report did not include any individual or group food consumption data. According to the study authors, food consumption was similar in controls and low- and mid-dose groups throughout the study. In the mid-dose animals, only one female (No. 1217) consumed 2% less food than the controls over the entire study period. A compound-related decrease in food intake in the high-dose males and females varied between 3.5-35%. One high-dose male (No. 1204) and one high-dose female (No. 1228) sacrificed moribund during the study consumed 31 and 35% less food than controls, respectively.

E. Hematology: Examination of the group mean data indicated apparent dose-related trends for a decrease in red blood cells (RBCs), hematocrit (HCT), and hemoglobin (Hb) in both males and females at months 1, 2, and 3. At months 4 and 5, these values were lower than control (although not necessarily significantly) in all dose groups of males and females; dose trends were not as evident. At months 1, 2, 3, and 4, RBCs were significantly ($p \leq 0.05$) decreased in all dosed males and in mid- and high-dose females; many of these changes were accompanied by significant ($p \leq 0.05$) decreases in Hb. At months 5 and 6, RBCs and Hb were significantly decreased in high-dose males and females only. Platelet counts were significantly increased ($p \leq 0.05$) in high-dose groups at most intervals (males and females at months 1, 2, 4, 5, and 6) and in mid-dose groups at several intervals (females at months 1, 3, 4, and 5; males at months 1, 2, 3, and 4). Reticulocyte counts were consistently slightly increased in dosed groups; however, no values reached a level of significance ($p \leq 0.05$) and all values were within the normal range for beagle dogs (<1.2%). Total leukocyte counts were slightly but significantly increased ($p \leq 0.05$) in mid- and high-dose females but only at 6 months. There were no changes in differential counts. Table 3 summarizes selected hematology data at 8 weeks and 6 months. From the early part of the study, Heinz and Jolly bodies appeared in RBCs of mid- and high-dose animals. An increase in reticulocytes and occurrence of Jolly bodies were reported to be dose-related incidences. Bone marrow examination in the high-dose animals at 6 months indicated possible impairment in hematopoiesis and thrombocytopoiesis. With the exception of one high-dose male (No. 1203) RBC values returned to normal in all dogs after the recovery period.

F. Clinical Chemistry: There were dose-related increases in alkaline phosphatase in females at months 1 through 6 (Table 4). The increases were significant for high-dose females at all six intervals for mid-dose females at months 2, 5, and 6 and for low-dose females at months 3 and 6. Increases in male groups

TABLE 3. Selected Mean Hematology Values (± SD) in Dogs Fed Different Levels of Trifluralin for 6 Months[a]

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Parameters Measured[b]	8 Weeks Dose (ppm) 0	400	1000	2500	6 Months Dose (ppm) 0	400	1000	2500
MALES								
Hb (g/dL)	15.37 ±1.19	13.97* ±0.58	12.48* ±0.90	10.95* ±1.23	14.05 ±1.52	12.67 ±1.17	13.35 ±1.19	11.72** ±1.03
RBCs (10^6/mm^3)	6.507 ±0.491	5.807* ±0.241	5.295* ±0.357	5.015* ±1.431	6.050 ±0.533	5.342 ±0.469	5.667 ±0.537	4.472** ±0.414
HCT (%)	44.5 ±3.3	41.2 ±1.2	38.5 ±2.7	33.3 ±4.2	40.8 ±4.2	38.0 ±4.0	40.8 ±2.7	36.0 ±3.3
L (10^3/mm^3)	8.35 ±2.78	8.53 ±1.74	8.83 ±1.11	8.05 ±2.88	7.00 ±1.38	8.03 ±2.61	8.78 ±1.16	8.08 ±2.20
R (%)	0.18 ±0.10	0.28 ±0.00	0.68 ±0.47	0.50 ±0.39	0.20 ±0.17	0.25 ±0.10	0.80 ±0.42	0.66 ±0.39
T (10^3/mm^3)	337.8 ±80.8	475.0* ±47.1	481.2* ±58.7	509.0* ±64.8	315.0 ±59.3	420.5 ±70.5	448.5 ±24.2	574.6* ±116.3
FEMALES								
Hb (g/dL)	15.87 ±0.65	15.03 ±0.80	13.77* ±0.65	±11.62* ±1.47	14.17 ±1.42	14.08 ±1.40	13.68 ±0.95	12.00 ±0.74
RBC (10^6/mm^3)	6.688 ±0.354	6.067 ±0.417	5.613* ±0.284	4.726 ±0.586	5.938 ±0.430	5.965 ±0.587	5.623 ±0.411	4.984* ±0.291
HCT (%)	46.0 ±2.3	42.8 ±3.4	42.0 ±3.0	36.4 ±6.8	41.2 ±3.1	41.3 ±4.0	40.2 ±2.5	36.4 ±2.1
L (10^3/mm^3)	9.77 ±1.84	11.65 ±2.50	12.28 ±2.64	9.78 ±1.71	8.43 ±1.74	8.35 ±0.71	11.08** ±0.99	11.88** ±1.49
R (%)	0.20 ±0.14	0.18 ±0.10	0.20 ±0.20	0.50 ±0.50	0.23 ±0.10	0.20 ±0.17	0.27 ±0.00	0.58 ±0.36
T (10^3/mm^3)	340.2 ±43.3	451.7 ±76.5	467.8 ±93.7	550.6* ±116.3	354.7 ±83.3	428.8 ±38.3	520.3 ±53.8	702.6* ±243.9

* Significantly different from control ($p \leq 0.05$) by ANOVA, followed by a multiple comparison procedure by Nemenyi.

** Significantly different from control ($p \leq 0.05$) by ANOVA, followed by a multiple comparison procedure by Scheffe.

[a] All means are based on six determinations, except for 2500-ppm groups where means are based on five determinations.

[b] Abbreviations used: Hb, hemoglobin; RBCs, erythrocytes; HCT, hematocrit; L, leukocytes; R reticulocytes; T, thrombocytes.

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TABLE 4. Serum Alkaline Phosphatase Activity (Mean ± SD; μ/L) in Female Dogs Fed Trifluralin

Interval (Month)	Dose Level (ppm)			
	0	400	1000	2500
0	165.33 ±30.87[a]	191.83 ±52.17	172.33 ±49.19	170.00 ±20.68
1	141.50 ±27.2	224.17 ±92.10	234.83 ±92.72	312.33* ±100.27
2	142.00 ±27.14	247.67 ±101.46	261.00* ±98.21	313.60* ±66.08
3	129.33 ±15.42	256.83* ±107.22	243.50 ±78.05	297.20* ±56.46
4	111.17 ±16.12	241.00 ±100.14	239.17 ±101.85	290.40* ±51.64
5	129.33 ±12.31	276.83 ±114.95	300.00* ±115.42	386.60* ±92.93
6	116.17 ±25.61	236.00 ±101.88	285.33* ±96.55	356.80* ±99.33

*Significantly different from control value ($p \leq 0.05$).

that were significant were sporadic. There were dose-related decreases in serum glutamic-pyruvic transaminase (SGPT) but not serum glutamic-oxaloacetic transaminase (SGOT) (see Section 14, Discussion). Changes in other clinical chemistry parameters that reached a level of significance ($p < 0.05$) were sporadic and all values were reported within the normal range of biological variation for beagle dogs. Results for methemoglobin were negative in all groups. Tests for hepatic and renal function gave similar results in dosed and control groups.

G. Urinalysis: None of the determinations showed any biologically significant differences. Urine from mid- and high-dose animals that was brown yellow to reddish brown was attributed to the intense reddish orange color of trifluralin.

H. Organ Weights: Mean absolute liver weights in low-, mid-, and high-dose males were significantly ($p \leq 0.05$) increased by 40, 56, and 40%, respectively; low-, mid-, and high-dose females showed 23, 41, and 64% increases over controls (Table 5). At the end of the recovery period, one high-dose male (No. 1199) showed an increased (639 g) liver weight. No other statistically significant changes in mean absolute organ weights were seen. Mean relative liver weights were significantly increased over control values in low-, mid-, and high-dose males (31, 58, and 75%, respectively) and females (22, 45, and 81%, respectively). Organ-to-body weight ratios of kidney in high-dose males (40%) and females (21%) were also increased (nonsignificantly). Absolute mean weight of testes in high-dose males was increased by 15%; however, in three high-dose males, testes were smaller than normal. In the report, statistical analyses for relative organ weights were performed on the combined male and female data and a significant ($p \leq 0.05$) increase in the kidney-to-body weight ratio was noted.

K. Gross Pathology: Table 6 summarizes gross findings. Enlarged livers and discolored kidneys were observed in all dosed groups. Gallbladders in two high-dose males and one high-dose female were filled with black gravelly contents. The majority of the mid- and high-dose males had smaller than normal testes and prostates. A yellowish discoloration of fatty tissues was found in all the high-dose and one mid-dose female. Hemorrhages in the region of right cardiac auricle were frequently observed in the low- and high-dose animals and hemorrhages in the rectum and colon were frequently observed in mid-dose animals. Two high-dose males were reportedly anemic.

After recovery, only one high-dose (male) dog had an enlarged liver; however, the kidneys in most of the animals remained darkly colored. No severe hemorrhages were observed.

TABLE 5. Mean Absolute (g ± SD) and Relative (% of Body Weight ± SD) Organ Weights in Dogs Fed Trifluralin for 6 Months[a]

Dose (ppm)	Spleen		Liver		Kidneys		Testes	
	Absolute	Relative	Absolute	Relative	Absolute	Relative	Absolute	Relative
				MALES				
Control	31.75 ±11.15	0.237 ±0.066	447.50 ±46.86	3.38 ±0.31	54.50 ±3.42	0.412 ±0.023	18.500 ±2.887	0.139 ±0.017
400	54.50	0.392 ±0.149	627.75* ±13.82	4.43* ±0.44	61.25 ±2.63	0.433 ±0.058	21.000 ±2.160	0.148 ±0.017
1000	53.75 ±26.30	0.408 ±0.193	699.00* ±67.76	5.34* ±0.43	56.75 ±9.32	0.432 ±0.060	17.000 ±1.414	0.130 ±0.010
2500	51.33 ±25.93	0.455 ±0.142	626.67* ±46.31	5.91* ±1.21	60.67 ±9.61	0.576 ±0.172	15.667 ±2.887	0.144 ±0.008
				FEMALES				
Control	49.000 ±13.491	0.386 ±0.114	446.50 ±50.72	3.50 ±0.43	51.00 ±3.56	0.399 ±0.034		
400	72.500 ±9.260	0.562 ±0.053	550.00* ±23.11	4.28* ±0.29	56.50 ±3.11	0.440 ±0.042		
1000	77.000 ±27.430	0.615 ±0.208	629.75* ±23.39	5.08* ±0.45	53.00 ±2.94	0.427 ±0.032		
2500	94.330 ±24.580	0.813 ±0.198	732.00* ±74.00	6.34* ±0.75	55.67 ±3.22	0.482 ±0.036		

[a]Organs from four dogs/sex were weighed in 0-, 400-, and 1000-ppm dose groups and from three dogs/sex in the 2500-ppm groups.

* Significantly different from controls ($p < 0.05$) (by ANOVA followed by Duncan's test for multiple comparisons; analyses were performed by our reviewers.

TABLE 6. Summary of Macroscopic Observations in Dogs Fed Trifluralin for 6 Months

Organ/Lesion	Dose Level			
	Control	400 ppm	1000 ppm	2500 ppm
		MALES		
	4[a]	4	4	3
Liver				
Enlarged	0	4	4	3
Kidneys				
Discolored	0	4	4	2
Gallbladder				
Black gravelly contents	0	0	0	2
Testes				
Smaller than normal	0	0	2	2
Prostate				
Smaller than normal	0	0	2	2
		FEMALES		
	4	4	4	3
Liver				
Enlarged	0	4	4	3
Kidneys				
Discolored	0	4	4	3
Gallbladder				
Black gravelly contents	0	0	0	1
Fatty Tissues				
Yellowish discolored	0	0	1	3

[a]Numbers of animals examined are underlined.

One high-dose female (No. 1228) sacrificed during the study showed changes in the basal region of the right cardiac auricle; multiple nodular thickenings in the subcapscular nodule; and liver with sharp edges, light color, and lobular markings. One high-dose male (No. 1204) sacrificed during the study showed severe atrophy of the prostate and of both testes and dark-color kidneys.

L. Histopathology: There were no distinct or consistent histologic alterations in the livers of dosed males and females that could be related to the increased organ weight.

Histopathologic examination of the three high-dose females sacrificed at 6 months revealed myocardial and hepatocytic fatty changes and Kupffer cell siderosis and pigments in the epithelium of the proximal convoluted tubule of the kidneys. Three high-dose males showed yellowish brown pigment in liver cells, diffused Kupffer cell siderosis in varying degrees, and pigmented epithelia of the proximal convoluted tubule of kidneys. The high-dose dogs (two/group) sacrificed after a month of recovery showed fatty changes of the myocardium (one male); pigment in liver cells (two males); slight liver congestion (two females); Kupffer cell siderosis in varying degrees of severity (two males, one female); abundant lymphoid follicles in the mucosa of the gallbladder (two females); and disseminated areas of calcification in the glandular mucosa of the stomach (one male, one female).

Histopathologic examination of one high-dose female (No. 1228) sacrificed during the study showed moderate myocardial fatty changes; periarteritis in the coronary region; fatty changes of the liver cells; slight Kupffer cell siderosis; confluent necrotic foci in the spleen; and periarteritis at the cortico-medullary junction of the kidney. Microscopic examination of the high-dose male (No. 1204) sacrificed during the study revealed testicular atrophy with azoospermia; marked prostatic fibrosis; flat gastric glandular mucosa; and pigments in the liver cells and proximal tubule of kidneys.

Impairment of hematopoiesis occurred in four dogs in the high-dose group. In all four mid-dose males, liver cells had foamy cytoplasm in the center of the lobules. Fat-free vacuoles were observed in the liver cells and zona glomerulosa of the adrenal cortex was seen in some females. All male and female kidneys showed deposits of fine-grain pigments in the epithelia of proximal convoluted tubules. Additional histologic findings included subepicardial and perivascular lipopexia (one male); fatty changes of the liver cells (one male, one female); and impaired spermatogenesis with azoospermia and prostatic fibrosis in one male.

In general, recovery animals of this dose group showed fat-free vacuoles and pigments in the liver cells, slight Kupffer cell siderosis, fatty changes in the myocardium, and pigments in the epithelia of the proximal convoluted tubule.

Individual control animals including those killed after recovery showed pigment deposits in the kidney tubules, slight Kupffer cell siderosis, fatty changes and fat-free vacuoles in the liver cells, and centrilobular hyperemia of liver.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded the following:

1. Food consumption and body weights for both the sexes at the 2500-ppm level were lower than for the control and other dosed groups.

2. One high-dose female after 39 days of dosing and one high-dose male after 85 days of dosing were sacrificed because of extremely low food consumption and deteriorating health.

3. Animals in all dose groups developed corneal lesions of traumatic origin. The frequency and severity of these lesions were increased with dose level. It was stated that trifluralin increased the severity of lesions or disturbed the process of healing.

4. All dosed animals developed toxico-hemolytic anemia, and the severity of the incidence was dose related. Thrombocyte values were also increased in all dosed groups.

5. The activity of serum alkaline phospnatase was increased in all dosed animals.

6. Enlarged livers were observed in all dosed animals; however, the substantial increase in the organ weight could not be matched with the histopathologic examination, indicating the presence of a cytotoxic effect.

7. Impairment of hematopoiesis was observed in four 2500-ppm dogs.

8. A NOEL was not achieved.

B. A quality assurance statement was signed and dated October 30, 1981.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

Under the conditions of the study, trifluralin was toxic to beagle dogs. This conclusion was based on the following compound-related effects observed at all dose levels: enlarged livers, discolored kidneys, corneal vascularization, hemolytic anemia, and increased serum alkaline phosphatase activity. Aversion to food caused starvation in two high-dose dogs; before being sacrificed moribund, food consumption in these dogs was 31-35% less than controls.

We agree with the study authors that toxic effects occurred at all dose levels; therefore, a NOEL was not achieved and the MTD was exceeded.

The report authors indicated an increase in reticulocyte count; however, no values reached a level of significance ($p \leq 0.05$) and none were above the normal range. A dose-related decrease in SGPT has no toxicologic importance; it may have been caused by a technical error or interference in the assay; SGOT did not show a similar effect.

The experimental design was inadequate for assessing the systemic toxicity of the test compound. Initially, there were six dogs/group, out of that three to four dogs/group were sacrificed after 6 months of dosing. Two dogs/group were kept on control diet for a further period (recovery) of 4 weeks for observation and then sacrificed. Four animals per treatment group in the major phase was not an adequate number to draw any definite conclusion from this study. After loss of two animals during the first-half of the study, only three animals/group remained in the 2500-ppm groups; statistical analyses could, therefore, not be made. The recovery phase consisted of only two dogs/sex/group; therefore, hardly any biological significance could be attributed to data from this part of the study.

Only initial and terminal body weights were provided in the summary table. Weekly body weights were presented graphically so the exact figures could not be determined. It was stated in the report that there were intergroup differences in food consumption, but no individual or summary data were provided to support this statement. Water consumption was not determined. Male and female data for corneal lesions were pooled together in the summary table; from the individual data presented it was not possible to separate the long-lasting lesions from the temporary lesions. Summary tables for the gross and histopathologic findings were not provided in the report.

The statistical treatments of data are not clear. In the materials and methods section of the study, statistical methods were referred to only in general terms. None of the individual or summary data tables contained levels of significance or dose-trend analysis. Instead, computerized summaries of statistical analyses were provided in a separate section. The clinical chemistry and hematology data were analyzed by ANOVA, followed by one of the multiple comparison

procedures of Nemenyi, Scheffe, or Tukey, or by Chi-square using a row by column contingency table. No reason for using any particular method was given, nor did the authors explain why the nonparametric Chi-square was used instead of the parametric ANOVA. For statistical analyses of organ weights, male and female data were pooled. These data should have been treated separately.

Some major deficiencies of this study are as follows:

1. Only four animals/sex/group were used for the major phase of the study, and one male and one female receiving 2500 ppm were sacrificed prematurely. According to EPA guidelines for long-term studies involving nonrodents, there should be no fatalities.

2. No summary tables for weekly body weights were provided; and no summary or individual food consumption data or water consumption data were provided.

3. Concentration, homogeneity, and stability of the test compound in the diets were not determined.

4. A LOEL and NOEL were not established, and the MTD was exceeded.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5-13.

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APPENDIX A

Materials and Methods

Trifluralin toxicology reviews

Page _____ is not included in this copy.

Pages 148 through 156 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

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EPA: 68-02-4225
DYNAMAC No. 208-B
August 8, 1986

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DATA EVALUATION RECORD

TRIFLURALIN

Chronic Feeding Study in Dogs

STUDY IDENTIFICATION: Bathe, R. 12-Month oral toxicity (feeding) study with trifluralin, substance technical grade (code HOE 38474 OH AT210) in beagle dogs. (Unpublished study No. A29701 prepared by Research and Consulting Co. AG, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated November 9, 1984.) Accession No. 259001.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 8-8-86

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1. CHEMICAL: Trifluralin.

2. TEST MATERIAL: Technical grade trifluralin, code: HOE 38474 OH AT210, contained greater than 99 percent active ingredient and was described as an orange crystal.

3. STUDY/ACTION TYPE: Chronic feeding study in dogs.

4. STUDY IDENTIFICATION: Bathe, R. 12-Month oral toxicity (feeding) study with trifluralin, substance technical grade (code HOE 38474 OH AT210) in beagle dogs. (Unpublished study No. A29701 prepared by Research and Consulting Co. AG, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated November 9, 1984.) Accession No. 259001.

5. REVIEWED BY:

William L. McLellan, Ph.D.
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Date: 8-8-86

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. When trifluralin was fed to dogs for 1 year at dietary levels of 30, 150, or 750 ppm, there was a decreased weight gain in males and females receiving 750 ppm. There were some significant ($p \leq 0.05$) decreases in red blood cell (RBC) parameters in high-dose males and females and an increase in methemoglobin. Total serum lipids, triglycerides, and cholesterol were increased in high-dose males and females when compared to controls. There were increases in liver weight in males receiving 150 and 750 ppm and females receiving 750 ppm trifluraiin and increases in mean spleen weight in females receiving 750 ppm. There were no histologic findings that correlated with the organ weight changes. Based on the increases in liver weights the LOEL is 150 ppm and the NOEL is 30 ppm.

B. The study is Core Guideline.

Item 8--see footnote 1.

9. BACKGROUND: Dose selection for this study was based on a subchronic feeding study in beagle dogs.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for complete details.)

1. The test substance, HOE 38474 OH, lot AT210, 99 percent pure, was an orange crystal stated to be stable for 2 years. Diets containing 30, 150, or 750 ppm test material were prepared 2 times a month by mixing the appropriate amount of test material with microgranulated dog food, repelleting after addition of water (10% v/w), and air drying. Diets were stored at 4°C and analyzed for stability and homogeneity and content of test substance at seven intervals during the study.

2. Pedigreed beagle dogs (source: Marshall Research Animals, N. Rose, NY), which were 21-30 weeks of age and dewormed and vaccinated, were examined for health and acclimated to the laboratory for 11 days. Males weighed 5.7-9.1 kg and females 5.1-6.9 kg. Randomly selected groups of six males and six females were fed diets containing 0, 30, 150, or 750 ppm trifluralin. Food (250 g/dog for the first 92 days and 300 g thereafter) was offered to the dogs for 3 hours/day; water was provided ad libitum. The dogs were housed individually in environmentally controlled kennels with a 12-hour dark/light cycle.

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[1] Only items appropriate to this DER have been included.

3. The dogs were observed twice daily for clinical signs of toxicity, behavioral changes, and mortality. Ocular examinations and hearing tests were conducted on all animals prior to dosing and at 1, 3, 6, 9, and 12 months. Individual food consumption was measured daily and body weights were recorded weekly.

4. Clinical laboratory analyses were conducted on all dogs at pretest and at 1, 2, 3, 6, 9, and 12 months. The hematologic parameters examined were RBC, hemoglobin (HB), hematocrit (HCT), platelet, reticulocyte, methemoglobin, and total and differential leukocyte counts; Heinz bodies and Howell-Jolly bodies were determined on appropriately stained blood smears; red cell morphology was microscopically examined; and coagulation time was determined. Twenty biochemical parameters were measured. Bromosulfophthalein clearance for hepatic function and phenolsulfophthalein clearance for renal function were performed on all dogs prior to the end of the study. Bone marrow smears were examined for control and high-dose dogs at study termination. Ten urinary parameters were conducted on samples collected by catheterization.

5. All surviving dogs were sacrificed by injection of euthanasia solution T61 (Hoechst) and complete gross examinations were performed. The following organs were weighed: brain, heart, liver, kidneys, testes, ovaries, pituitary, thyroid, adrenals, and spleen. Approximately 42 tissues were fixed in formalin, sectioned, and stained for histopathologic evaluation.

6. Body weights, food consumption, organ weights, and clinical laboratory data were analyzed using univariate one-way analysis of variance, followed by Student's t-test for intergroup comparisons. Williams' test was used to determine the lowest group significantly different from control if there was a dose-response trend. Fisher's exact test (2 x 2 tables) was used for appropriate data.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

Dietary Analysis: The mean analyzed concentrations of trifluralin in the diets (measured at 0, 13, 27, and 41 weeks) were 86.6±11, 95.0±10.1, and 93.7±9.0 percent of the nominal values at dose levels of 30, 150, and 750 ppm, respectively. Analysis showed that the test compound was homogenous in the diets and that it was stable for 21 days when refrigerated.

Clinical Observations and Mortality: A female dog receiving 150 ppm died on day 83, and a male receiving 750 ppm was sacrificed moribund on day 142. Both deaths were considered unrelated to dosing. One male (No. 22) receiving 750 ppm was diagnosed with chronic myositis and was treated with corticosteroid and antibodies. The animal survived to termination.

Incidents of diarrhea were observed in all animals. Occurrence was more frequent in high-dose males during weeks 1-41 and high-dose females throughout the study. Diarrhea was severe in one control male, one male and one female receiving 150 ppm, and one male receiving 750 ppm. Other clinical signs of toxicity, blood and/or mucus in feces, spontaneous vomiting, swollen mammae, and alopecia were observed in some dogs but they were not considered compound related.

No compound-related effects were found in hearing tests. Ophthalmoscopic findings were similar in all groups of dogs. Small opacities were seen on the lens of two dogs: a control female and a female receiving 150 ppm, and a small nodule on the cornea was seen in one male receiving 750 ppm. Occasional redness of the conjunctivae was seen in one male and one female control, four males and three females receiving 30 ppm, and two males and four females receiving 750 ppm. These findings were considered incidental.

Body Weights and Food Consumption: It was reported that mean body weight gain was lower than controls in high-dose males from week 18 onward and in high-dose females during weeks 17-44. The mean weight gains in males and females receiving 750 ppm trifluralin were 76.4 and 72.3 percent of the control values, respectively. There were no statistically significant ($p < 0.05$) effects on body weights. Data for selected intervals are summarized in Table 1. No effects were seen at doses of 30 and 150 ppm. There were sporadic intervals of decreased food consumption but mean values were similar in all groups.

Hematology: It was reported that there were no changes of toxicologic importance in hematology parameters at any interval of analysis during the study. All values, with the exception of methemoglobin, whether or not significantly different from the control values, were within the range of values (95% confidence limits) normally found in historical control groups for the laboratory. These historical data were provided for up to 280 animals/sex. Statistical significance ($p < 0.05$ or < 0.01) was found for decreased RBC and HB in high-dose males at months 3, 6, and 12 but not month 9; for RBC in high-dose females at months 1, 2, 3, and 9 but not 6 or 12; and HB in high-dose females at months 1 and 2 but not at other intervals. No Heinz bodies were found in RBC, and bone marrow smears showed normal differentiation. Mean data for methemoglobin are summarized in Table 2. The significant values are above the normal range for beagle dogs.

TABLE 1. Selected Mean Body Weights (g) and Body Weight Gains (g) of Dogs Fed Trifluralin for 1 Year

Dose Level (ppm)	Group Mean Body Weight (± S.D.)[a] at Week				Mean Total Weight Gain[b] from Week 0 to 52
	Pretest	13	26	52	
			Males		
0	7409±1171.5	7908± 903.2	9218± 779.6	9756± 707.4	2347±1617.8
30	7167±1321.1	8053± 934.1	8996±1064.7	9858±1422.1	2691±1028.4
150	7703± 817.8	8206± 972.7	9146±1459.6	9814±2040.9	2111±2522.4
750	7313± 853.7	7843± 614.5	8475± 748.0	9171± 695.3	1793± 567.3
			Females		
0	5750± 479.4	5619± 774.7	7972± 854.0	9249±1435.4	3499±1279.2
30	6389± 352.1	6993± 639.5	8261± 967.4	9488±1139.6	3098±1329.0
150	6078± 336.6	7490± 364.5	8812± 781.0	9818±1335.5	3743±1378.6
750	6014± 451.1	7217± 480.0	8184± 735.9	8545±.645.5	2531± 842.3

[a]Based on six animals/sex/group, except in the 750-ppm males (weeks 26 and 52 and total weight gain) and the 150-ppm females (all weeks and total weight gain), which included five animals/sex/group.

[b]Calculated by the reviewers.

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TABLE 2. Mean Methemoglobin Data for Dogs Fed Trifluralin for 1 Year

Dose Level (ppm)	Group Mean Methemoglobin Values (%) after Month					
	1	2	3	6	9	12
			Males			
0	0.8	0.7	0.7	1.0	0.8	1.0
30	0.7	0.7	0.6	1.1	0.8	0.9
150	0.9	0.8	0.9	1.5*	1.2*	1.4
750	1.1	1.3**	1.3**	1.9**	1.6**	1.9**
			Females			
0	0.8	0.8	0.7	1.1	1.0	1.1
30	0.7	0.8	0.8	1.3	1.2	1.4
150	0.8	0.9	1.0	1.4	1.3	1.8*
750	1.3**	1.5**	1.3**	2.1**	1.9**	2.4**

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

The author stated that there were no changes of toxicologic importance in biochemical data. There were significant increases compared to control in the level of triglycerides, total cholesterol, and total lipids in both high-dose males and females at most intervals of measurement during the study (Table 3). A slightly but significantly ($p = 0.05$) lowered albumin/globulin ratio was found in high-dose males after 1, 2, and 6 months and in high-dose females after 1, 2, and 3 months. All mean values were within the range of age-matched historical laboratory controls.

Urinalysis parameters were similar in control and dosed groups.

Organ Weights: Mean organ weight data (absolute and relative comparisons to body or to brain weights) for liver and spleen are presented in Table 4. Absolute mean liver weight but not relative weight was increased ($p \leq 0.05$) in males receiving 150 ppm. Absolute mean liver weights and weights relative to body or brain weights were significantly increased ($p \leq 0.01$) in both males and females receiving 750 ppm when compared to the appropriate control. The increase in absolute liver weight was 41 and 29 percent for high-dose males and females, respectively. Spleen weight was increased ($p \leq 0.05$) in high-dose females and spleen-to-body weight ratios were increased ($p \leq 0.05$) in high-dose dogs of both sexes. The increased spleen weight in high-dose males may have been caused by a value of 71.39 g in male No. 22. If this value is censored, the mean would be 28.85±7.32. This dog had splenomegaly and hepatomegaly and had been treated with corticosteroids for myositis during the study. Other organ weights in dosed groups were similar to controls.

Gross Necropsy: The high-dose male that was sacrificed moribund at day 141 had a unilateral epididymal enlargement and darkened areas of the colon. The female receiving 150 ppm that died on day 83 had copious exudation into the pleural cavities, pleural adhesion, and a distinct lobular pattern in the liver. In one high-dose male sacrificed at study termination there was hepatomegaly, splenomegaly, and yellow discoloration of body fat. Other findings, particularly lesions in the lungs (foci) and spleen (hematomas) and intestinal nematodes, occurred randomly.

Histopathology: The high-dose male that was sacrificed *in extremis* had myocardial necrosis with fatty changes, pericarditis/epicarditis, and arteritis in the bladder and epididymis; bone marrow showed myeloid hyperplasia and erythroid hypoplasia. The female receiving 150 ppm that died had pleuropneumonia of the lungs, liver necrosis and fatty changes, and bone marrow atrophy.

A high-dose male that had been treated with corticosteroids had amyloidosis in several tissues, vacuolar swelling and some fatty changes in the liver, and cortical atrophy in the adrenal. This dog also had bone marrow findings, erythroid hypoplasia, and moderate myeloid hyperplasia. The finding of amyloid may have been compound related. Other dogs had a few random incidental findings that were not considered compound related. There were no histologic findings that correlated with increased spleen and liver weight in high-dose animals.

TABLE 3. Selected Mean Clinical Chemistry Data for Dogs Fed Trifluralin for 1 Year

	Dose (ppm)/Male				Dose (ppm)/Female			
Parameter/Month	0	30	150	750	0	30	150	750
Total Lipids (g/L)								
1	7.1	6.7	6.4	10.0**	6.7	6.3	7.2	8.8*
2	7.0	6.0	6.4	9.5**	6.5	6.4	7.1	8.5*
3	6.5	5.6	5.4	8.9**	6.2	5.9	6.3	8.7**
6	7.7	7.0	6.2	11.9**	8.4	7.4	8.2	12.0**
9	6.0	5.9	6.0	8.5**	7.1	6.6	7.2	9.4*
12	6.0	5.9	5.9	9.0**	8.5	8.3	8.0	12.7**
Total Cholesterol (mmol/L)								
1	4.39	3.94	3.56*	5.77**	3.68	3.46	3.88	5.23**
2	4.91	4.32	3.86	6.28**	3.98	3.90	4.51	5.46*
3	3.47	3.05	2.75	4.61*	3.09	2.81	2.96	3.97
6	3.85	3.36	3.05	6.05*	4.17	3.62	4.04	6.21**
9	3.31	3.22	3.03	4.56**	4.04	3.37	3.80	4.94
12	3.34	3.17	3.16	4.95**	4.42	4.24	4.09	6.59*
Triglycerides (mmol/L)								
1	0.40	0.36	0.40	0.62**	0.41	0.37	0.37	0.46
2	0.52	0.45	0.47	0.70*	0.43	0.43	0.47	0.68**
3	0.42	0.33	0.36	0.53*	0.36	0.35	0.34	0.48*
6	0.38	0.38	0.37	0.54*	0.46	0.32	0.43	0.59
9	0.36	0.40	0.41	0.52*	0.36	0.39	0.47	0.50*
12	0.43	0.35	0.36	0.52	0.54	0.42	0.47	0.52

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

TABLE 4. Selected Mean Organ Weights (± S.D.),[a] Organ-to-Body Weight Ratios, and Organ-to-Brain Weight Ratios of Dogs Fed Trifluralin for 1 Year

Dose Level (ppm)	Organ Weight (g)		Organ/Body Weight (%)		Organ/Brain Weight (%)	
	Liver	Spleen	Liver	Spleen	Liver	Spleen
			Males			
0	291.4 ±15.2	24.29 ±4.61	3.188 ±0.310	0.263 ±0.033	362.827 ±36.803	29.935 ±4.185
30	294.3 ±22.6	26.73 ±5.59	3.213 ±0.429	0.288 ±0.046	361.213 ±13.116	32.583 ±4.945
150	318.0* ±21.2	28.07 ±12.70	3.585 ±0.825	0.290 ±0.080	396.363 ±42.120	34.346 ±14.678
750	412.0** ±23.5	37.36 ±20.05	4.633** ±0.181	0.423* ±0.206	529.353** ±38.468	50.250 ±30.483
			Females			
0	279.6 ±37.7	23.28 ±5.46	3.199 ±0.556	0.271 ±0.087	383.343 ±49.784	32.000 ±6.941
30	266.3 ±34.9	23.18 ±4.77	2.984 ±0.544	0.259 ±0.058	375.905 ±45.134	32.943 ±7.318
150	274.7 ±30.0	28.26 ±7.53	2.966 ±0.158	0.306 ±0.089	359.721 ±39.110	37.081 ±10.730
750	361.5** ±31.7	30.40* ±4.74	4.555** ±0.538	0.383* ±0.067	492.300** ±71.154	41.122 ±6.058

[a]Based on six animals/sex/group, except in the 750-ppm males which included four animals for liver weight and ratios and five animals for spleen weight and ratios, and in the 150-ppm females, which included five animals for both liver and spleen weight and ratios.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that the NOEL for the study was 150 ppm for male and female dogs, which corresponded to a compound intake of approximately 4.8 mg/kg/day. Body weight gains were decreased in males and females receiving 750 ppm. There were no toxicologically important changes in hematology parameters. Slight increases in lipids at 750 ppm suggest regulative and adaptive changes in lipid metabolism and since the findings are not supported by histopathologic findings, they may be of no toxicologic importance. There were statistical increases in mean liver weight in mid-dose males and in high-dose dogs of both sexes, and mean spleen weight was increased when compared to controls in high-dose males and females. The organ weight changes were not accompanied by any histologic correlates. The author concluded that the liver may be a target organ of toxicity.

B. A signed quality assurance statement was dated November 12, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study design was adequate and complete and the conduct of the study and reporting of data were acceptable. Historical reference values for clinical laboratory parameters were provided; these were useful in interpretation of the results of the current study. A few pages tabulating individual hematology and clinical chemistry data were missing in the report: pp. 289, 294, 304, 306, 316, and 364.

In general, we agreed with the report author's interpretation of the data. Although mean body weights were not significantly lower in males and females receiving 750 ppm when compared to controls a weight gain of 72-76 percent of controls is indicative of an effect; this was not accompanied by a decreased food consumption. It is our assessment that the increase in methemoglobin in high-dose males and females may have been compound related; the report author did not discuss the toxicologic significance. The increase in serum lipids in high-dose males and females may be a reflection of effects on the liver.

Since there was a significant increase in the absolute and relative mean liver weights in males receiving 150 and 750 ppm and sporadic increases in methemoglobin at 150 ppm, it is our assessment that the LOEL for the study is 150 ppm and the NOEL is 30 ppm trifluralin. The study author set the NOEL at 150 ppm.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 14-28.

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APPENDIX A

Materials and Methods

Trifluralin toxicology reviews

Page _____ is not included in this copy.

Pages 169 through 183 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-02-4225
DYNAMAC No. 208-A
January 23, 1987

DATA EVALUATION RECORD

TRIFLURALIN

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Becker, H., Mueller, E., Ellgehausen, H., Westen, H., Schlotke, B., and Terrier, C. Multiple generation study with trifluralin substance technical grade (Code: HOE 38474 O H AT210) in the rat. (Unpublished project No. 008875 by Research & Consulting Company AG, Itingen, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated October 17, 1984.) Accession Nos. 258994-258995.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-27-87

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1. CHEMICAL: Trifluralin; α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine.

2. TEST MATERIAL: Trifluralin, technical grade, was described as a red solid containing greater than 99% active ingredient and stable at 25° or -5°C for 2 years.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Becker, H., Mueller, E., Ellgehausen, H., Westen, H., Schlotke, B., and Terrier, C. Multiple generation study with trifluralin substance technical grade (Code: HOE 38474 O H AT210) in the rat. (Unpublished project No. 008875 by Research & Consulting Company AG, Itingen, Switzerland, for Hoechst Aktiengesellschaft, Federal Republic of Germany; dated October 17, 1984.) Accession Nos. 258994-258995.

5. REVIEWED BY:

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Marcia Van Gemert, Ph.D.
EPA Reviewer, Section Head

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Date: 1/28/87

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7. CONCLUSIONS:

A. The NOEL for parental toxicity of trifluralin in rats could not be determined due to increased relative kidney weights at all dose levels tested (i.e., 200, 650, and 2000 ppm), renal lesions of the proximal tubules and increased relative liver weights at 650 and 2000 ppm, one death due to acute renal failure at 650 ppm, and reduced body weights at 2000 ppm. The LOEL for this study was 200 ppm.

Repro. Tox NOEL - 650 LOEL - 2000 Developmental NOEL - 200 LOEL - 650 MRID 401457

The NOEL and LOEL for reproductive and developmental toxicity were 200 and 650 ppm, respectively, based on reduced weanling body weights at 650 and 2000 ppm and reduced litter sizes at 2000 ppm.

B. This study is classified Core Minimum.

Items 8 through 10 --see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Technical grade trifluralin was described as a red solid with greater than 99% purity. The test material was mixed with granulated food at least every 2 weeks to produce concentrations of 0 (control), 200, 650, and 2000 ppm. The diets were pelleted and refrigerated in paper bags until used. Food pellets were analyzed for concentration, homogeneity, and stability of the test material in the diet preparations. Animals were fed their respective test diets throughout the study.

2. Animals and Experimental Design: Male and female outbred Wistar KFM-Han, SPF quality, rats were obtained from KFM Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland, housed individually, and acclimated for 10 days. At the age of 6-7 weeks, 30 males and 30 females were randomly assigned to each of four study groups and designated F_0 parental animals.

After receiving the test diets for 80 days, F_0 animals were paired, one male to one female of the same group, for up to 20 days to produce F_{1a} litters. Litters were weaned at day 21 postpartum and approximately 10 days later the adults were rebred (using different pairs) to produce F_{1b} litters. Where possible, animals that were not fertile after the first breeding were subsequently paired with fertile animals.

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[1]Only items appropriate to this DER have been included.

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Twenty-six male and 26 female F_{1b} weanlings per group were selected to be F_1 parental animals and received their respective diets for at least 100 days before breeding. F_1 adults were bred (siblings were not paired) to produce F_{2a} and F_{2b} litters using the same procedures described for their parents.

Body weights of parental animals were recorded weekly except during the breeding periods. Mated females were weighed on gestation days (GD) 0, 7, 14, and 21 and on days 1, 4, 7, 14, and 21 postpartum. Food consumption was recorded at the same intervals as body weights except the food consumption of dams was only recorded until day 14 postpartum.

Vaginal smears were examined daily during the breeding periods to detect mated females and estrous cycle anomalies. The day on which vaginal sperm or a vaginal plug were found was designated GD 0.

During late gestation, females were examined twice daily for signs of parturition; gestation lengths were recorded. The day that parturition was completed was considered day 0 postpartum. As soon as possible after parturition, litters were examined for live and dead pups and gross anomalies. Sex ratios were determined on days 0 and 21. Pups were identified individually by tattoos on day 1 and subsequently by color spots on the hair. Pups were individually weighed on days 1, 4, 7, 14, and 21.

3. Observations and Measurements: Animals were examined twice daily for mortality and clinical signs of toxicity. Animals found dead or euthanized were necropsied and tissues were saved for histological examination. Surviving parental animals were killed after 'b' litters were weaned and necropsies were performed. Selected organs were weighed and tissues were saved for possible histological examination. Kidneys of F_1 adults were examined histologically in all groups; other tissues were examined only in the control and high-dose groups.

The uteri of apparently nonpregnant females were stained with ammonium sulfide to detect implantation sites. The testes, prostate, and seminal vesicles of all F_1 males that were not proven fertile were also weighed and examined histologically.

Pups found dead were necropsied and/or preserved for possible further examination. Weanlings not selected to be F_1 parental animals were killed and examined macroscopically. One pup/sex/litter was selected for necropsy; organs were weighed and tissues were preserved for possible histological

examination. Tissues from the selected control and high-dose F_{2b} progeny were examined histologically. Organ weights for the F_{1a} and F_{1b} progeny were not compared since these litters were killed at various times between days 21 and 32 postpartum.

4. Statistical Methods: Univariate one-way analysis of variance (ANOVA) was used to assess body weight, food consumption, organ weight, and reproduction data "if the variables could be assumed to follow a normal distribution." Student's t-test, based on a pooled variance estimate, was used to identify significant differences from the control group. A univariate one-way ANOVA, based on Wilcoxon ranks, together with the Kruskal-Wallis test was used to assess the litter population data. Sex ratios were analyzed using a 2 x 2 chi-square test.

B. Protocol: A protocol was not included in the study report.

12. REPORTED RESULTS:

A. Test Material Analyses: Chemical analyses of the test diets revealed mean concentrations (± S.D.) of 93.3 ± 6.6, 95.0 ± 8.5, and 91.0 ± 4.3% of the nominal values for the low-, mid-, and high-dose levels. Homogeneity samples were within 8.5% for all groups. Stability assays indicated that the test material concentrations generally remained within 10% of initial values for 3 weeks; however, diets prepared on two of the eight dates tested showed greater than 20% declines in concentration over the 3-week period.

B. Parental Data: Deaths occurred in one mid-dose male of the F_0 generation and in one female from each of the control and mid- and high-dose groups of the F_1 generation. Acute renal failure was diagnosed as the cause of death for the F_1 mid-dose female; the study authors did not report diagnoses for the other deaths.

Yellow discoloration of the urine was the only clinical finding associated with the compound. Although this finding was reportedly dose related, no summary tables or individual clinical findings were included in the study report.

Body weights of F_0 parental animals revealed marginally significant ($p < 0.10$) reductions in low- and high-dose males (Table 1). No other significant weight reductions were noted in the F_0 generation. In the F_1 parental animals, however, high-dose female body weights were significantly ($p < 0.05$) reduced during all phases of the generation (Tables 1 and 2).

TABLE 1. Mean Body Weights (g) of Rats Fed Trifluralin Prior to Breeding

	Dose Level (ppm)	Pretest	Week 1	Week 4	Week 8	Week 12
F_0 Males	0	118	163	275	354	398
	200	121	164	269	342	383
	650	119	166	272	350	391
	2000	118	164	268	340	382
F_0 Females	0	99	126	174	206	222
	200	98	125	174	206	222
	650	101	128	172	202	217
	2000	100	126	168	197	212
F_1 Males	0		98	220	331	369
	200		98	226	338	378
	650		90	215	329	364
	2000		87	208	314	348
F_1 Females	0		91	156	202	221
	200		87	155	203	220
	650		82	150	198	218
	2000		77*	146*	190*	207*

*Significantly different from control value ($p < 0.05$).

TABLE 2. Mean Maternal Body Weights (g) of Rats Fed Trifluralin

	Dose Level (ppm)	Gestation Day 0	Gestation Day 14	Gestation Day 21	Lactation Day 1	Lactation Day 4	Lactation Day 14	Lactation Day 21
F_0 Females								
F_{1a} Interval	0	225	268	327	247	259	284	270
	200	223	264	347	242	258	283	272
	650	219	257	344	240	253	278	268
	2000	212	251	352	228	246	269	261
F_{1b} Interval	0	252	292	361	271	285	311	294
	200	251	288	354	266	285	310	302
	650	245	283	352	260	277	303	294
	2000	236	268	329	249	263	290	284
F_1 Females								
F_{2a} Interval	0	235	276	340	237	260	292	284
	200	226	265	325	236	254	288	278
	650	227	266	329	230	252	284	276
	2000	219*	255*	310*	218*	241*	270*	272*
F_{2b} Interval	0	262	301	367	279	294	320	305
	200	261	299	365	277	293	323	307
	650	253	291	357	271	288	313	299
	2000	240*	274*	334*	255*	271*	294*	289*

*Significantly different from control value ($p < 0.05$).

Food consumption was significantly reduced during the first week of the study for both F_0 males and females of the high-dose group (Table 3). Significantly reduced food consumption was also noted during week 3 and both lactation periods (Table 4) for the F_0 females. In the F_1 generation, food consumption of high-dose males was significantly reduced when compared to controls for a 2-week interval after the second breeding period. In general, high-dose females had significantly reduced food consumption during the second gestation and lactation periods. Food conversion ratios were similar in all groups for both sexes of both generations.

Gross necropsies of parental animals revealed yellow discoloration of adipose tissue in mid-dose females and high-dose males and females of both generations; high-dose females showed the greatest incidences. Histological examinations of F_1 adults also revealed dose-related increased incidences of lesions of the renal proximal tubules in mid- and high-dose females. In addition, increased incidences of hyaline droplets in the tubular epithelium occurred in females of all dosed groups and reduced incidences of corticomedullary mineralization occurred in mid- and high-dose females. All other gross and microscopic lesions were considered incidental.

Dose-related significant increases in relative liver weights occurred in mid- and high-dose F_0 males, F_1 males, and F_1 females and in high-dose F_0 females (Table 5). Significantly increased relative kidney weights occurred at all dose levels of F_0 males and in mid- and high-dose F_1 males. High-dose males of both generations and mid-dose males of the F_1 generation also had significantly increased relative testicular weights. Significantly reduced thymus weights occurred in high-dose males and females of the F_1 generation. Other significant differences in organ weights occurred inconsistently across generations or in nondose-related patterns.

Reproductive and Developmental Data: The proportions of females mating, becoming pregnant, delivering, and rearing their litters to weaning were generally comparable for all groups at all breeding intervals (Table 6). In addition, precoital intervals and gestation lengths were comparable for all groups and the behavior of dams during parturition and nursing were also reportedly similar for all groups.

High-dose litter sizes on day 0 were slightly reduced in the F_{1b} interval and significantly reduced in the F_{2a} and F_{2b} litters when compared to controls (Table 7). The F_{2b} high-dose litters were also significantly smaller on day 21. Pup mortality and pup weights at birth did not indicate any compound effects; however, mid-dose weanling weights were significantly reduced at all but the F_{2a} interval, and high-dose weights were significantly reduced at all litter intervals. External examinations of

TABLE 3. Mean Food Consumption (g/rat/day) of Rats Fed Trifluralin Prior to Breeding

	Dose Level (ppm)	Pretest	Week 1	3	7	10	11
F_0 Males	0	20	23	24	24	23	26
	200	20	23	24	23	23	22
	650	21	23	24	25	23	23
	2000	20	21*	23	24	23	23
F_0 Females	0	16	17	17	17	16	18
	200	16	16	17	16	16	16
	650	17	16	16	17	16	15
	2000	16	15*	15*	16	16	16
F_1 Males	0		19	25	26	28	25
	200		18	23	25	26	24
	650		18	23	26	26	24
	2000		19	22	24	27	24
F_1 Females	0		16	17	18	21	19
	200		15	17	18	19	17
	650		14	16	18	19	17
	2000		17	16	17	19	17

*Significantly different from control value ($p < 0.05$).

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TABLE 4. Mean Maternal Food Consumption (g/rat/day) of Rats Fed Trifluralin

	Dose Level (ppm)	Gestation Days			Lactation Days		
		0-7	7-14	14-21	1-4	4-7	7-14
F_0 Females							
F_{1a} Interval	0	19.5	21.1	22.2	29.4	40.0	55.0
	200	18.6	20.3	25.1	29.6	40.7	55.5
	650	17.8	19.7	26.0	33.3	39.4	55.7
	2000	18.7	20.1	27.5	28.5	37.1*	50.8*
F_{1b} Interval	0	19.5	21.4	21.7	36.5	47.0	62.1
	200	18.4	20.5	21.6	36.2	46.3	62.0
	650	18.4	20.6	22.2	38.8	48.7	63.0
	2000	18.5	20.8	20.7	38.5	42.4*	56.6*
F_1 Females							
F_{2a} Interval	0	19.0	21.4	22.8	31.9	47.7	58.3
	200	18.8	21.1	21.5	32.7	48.3	61.2
	650	19.6	21.3	22.1	33.7	49.9	60.0
	2000	19.4	20.8	21.5	32.5	45.8	57.5
F_{2b} Interval	0	20.8	23.3	24.0	38.4	49.7	66.0
	200	19.6	22.0	22.3	35.8	49.7	67.6
	650	21.0	22.0	21.2	37.5	46.3	63.9
	2000	19.3*	21.7	21.7*	33.4*	44.9*	59.1*

*Signicantly different from control value ($p < 0.05$).

TABLE 5. Mean Relative Organ Weights (% Body Weight) of Rats Fed Trifluralin

	Dose Level (ppm)	Body Weight (g)	Liver	Kidneys	Thymus	Gonads
F_0 Males	0	481	3.08	0.51	0.044	0.79
	200	465	3.17	0.54**	0.048	0.81
	650	473	3.27**	0.55**	0.045	0.8C
	2000	459	3.73**	0.58**	0.042	0.85*
F_0 Females	0	271	4.42	0.70	0.043	0.034
	200	273	4.55	0.69	0.044	0.035
	650	264	4.62	0.69	0.049	0.037
	2000	264	5.60**	0.72	0.045	0.032
F_1 Males	0	472	3.34	0.49	0.061	0.77
	200	486	3.44	0.50	0.059	0.80
	650	465	3.67**	0.54**	0.062	0.84*
	2000	446*	3.86**	0.54**	0.052*	0.87*
F_1 Females	0	269	4.04	0.64	0.094	0.040
	200	268	4.32	0.64	0.089	0.040
	650	265	4.42**	0.63	0.082	0.042
	2000	253**	4.90**	0.63	0.079*	0.040

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 6. Summary of Reproductive Performance of Rats Fed Trifluralin

	Dose Level (ppm)	No. Paired	No. Mated	Pregnant No.	Pregnant %	Delivering No.	Delivering %	Weaning No.	Weaning %
F_0 Females									
F_{1a} Interval	0	30	29	29	100	29	100	28	97
	200	30	30	28	93	28	100	27	96
	650	30	30	30	100	30	100	30	100
	2000	30	30	29	97	29	100	29	100
F_{1b} Interval	0	30	30	28	93	28	100	28	100
	200	30	30	27	90	27	100	27	100
	650	30	30	23	77	23	100	23	100
	2000	30	30	29	97	29	100	29	100
F_1 Females									
F_{2a} Interval	0	26	26	24	92	24	100	24	100
	200	26	26	20	77	20	100	20	100
	650	26	26	21	81	21	100	20	95
	2000	26	26	23	88	23	100	23	100
F_{2b} Interval	0	26	26	21	81	21	100	20	95
	200	26	26	25	96	25	100	25	100
	650	26	26	25	96	23	92	22	96
	2000	26	26	26	100	16	100	26	100

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TABLE 7. Summary of Litter Data of Rats Fed Trifluralin

	Dose Level (ppm)	Mean No. Live Pups[a] Day 0	Mean No. Live Pups[a] Day 21	% Mortality[a] Days 1-21	Mean Pup Weight (g) Day 1	Mean Pup Weight (g) Day 7	Mean Pup Weight (g) Day 21
F_{1a} Litters	0	10.4	10.1	2.7	6.2	12.9	41
	200	10.7	10.4	2.8	6.0	12.5	40
	650	11.0	10.7	2.7	6.1	12.1*	37*
	2000	10.2	9.8	3.7	6.1	11.9*	36*
F_{1b} Litters	0	11.9	11.5	3.3	6.2	12.7	38
	200	11.4	11.2	1.3	6.4	13.0	39
	650	12.0	11.8	1.4	6.2	12.1*	35*
	2000	10.7	10.2	4.8	6.0	12.0*	34*
F_{2a} Litters	0	11.8	11.3	4.2	5.8	12.1	39
	200	11.8	11.5	2.6	5.8	12.3	40
	650	11.7	11.4	2.1	5.9	12.2	38
	2000	10.3*	10.1	1.7	5.6*	11.6*	36*
F_{2b} Litters	0	12.0	11.8	1.7	6.4	13.2	41
	200	11.9	11.7	1.3	6.3	13.2	41
	650	11.4	11.3	0.8	6.5	13.1	40*
	2000	10.1*	10.1*	0.0	6.4	12.9	38*

[a]Does not include litters that did not survive to day 21.

*Significantly different from control value ($p < 0.05$).

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the pups revealed no anomalies attributable to the compound. Sex ratios of the pups also did not suggest a compound effect.

Organ weight comparisons of the F_{2a} and F_{2b} weanlings revealed significantly increased relative liver weights in the high-dose group at both litter intervals (Table 8). Significantly increased relative kidney weights were also noted by the study authors for F_{2b} females. Significantly increased relative testicular weights occurred at both intervals. Other significant changes did not appear to indicate an effect. No histological abnormalities attributable to the compound were noted.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors cited the NOEL of this study to be 200 ppm. They noted that there were slight parental effects at 650 and 2000 ppm, reduced litter sizes at 2000 ppm, and reduced pup weight gains at 650 and 2000 ppm.

B. A quality assurance statement was signed and dated November 14, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material Analyses: Chemical analyses of the test diets indicated that they were accurately and properly prepared. Although diets prepared on two dates showed greater than 20% declines in concentration after 21 days, these were isolated occurrences and were not regarded to affect the integrity of the study.

Parental Data: Deaths were infrequent and their overall incidence did not suggest a compound effect; however, we regard the death of a mid-dose F_1 dam (attributed to acute renal failure) to be compound related. The renal lesions observed in this animal were consistent with the microscopic changes seen in mid- and high-dose F_1 kidneys. We consider the occurrence of lesions of the renal proximal tubules to be toxic effects in females at 650 and 2000 ppm. Hyaline droplets in the tubular epithelium in females of all dose groups and the reduced incidences of corticomedullary mineralization in mid- and high-dose females were considered to be physiological responses rather than toxic effects.

We consider the following significant changes in relative organ weights to be compound related: increased liver weights in males and females at 650 and 2000 ppm, increased kidney weights in males at all dose levels, reduced thymus weights in females at 2000 ppm, and increased testicular weights at 650 and 2000 ppm.

TABLE 8. Mean Relative Organ Weights (% body weight) of Weanlings of Rats Fed Trifluralin

	Dose Level (ppm)	Body Weight (g)	Liver	Kidneys	Gonads	Uterus
F_{2a} Males	0	41	4.02	1.04	0.50	--
	200	42	4.08	1.04	0.52	--
	650	39	4.12	1.04	0.51	--
	2000	38	4.44**	1.05	0.54**	--
F_{2a} Females	0	38	3.88	1.06	0.044	0.107
	200	40	4.00	1.06	0.037**	0.103
	650	38	4.03	1.06	0.039*	0.101
	2000	37	4.38**	1.09	0.041	0.093
F_{2b} Males	0	43	3.98	1.00	0.48	--
	200	43	4.05	1.05*	0.49	--
	650	43	4.08	1.00	0.48	--
	2000	40	4.31	1.04	0.52*	--
F_{2b} Females	0	41	3.90	1.05	0.044	0.098
	200	41	3.98	1.07	0.041	0.101
	650	40	4.12*	1.08	0.044	0.102
	2000	38	4.26**	1.10**	0.042	0.100

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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We regard the increased relative kidney weights to be indicative of a toxic effect in F_0 males at 200, 650, and 2000 ppm and in F_1 males at 650 and 2000 ppm. In addition, increased relative liver weights indicated a toxic effect in parental animals of both generations at 650 and 2000 ppm. We consider the yellow discoloration of adipose tissue and urine to be compound related, but not to reflect overt toxicity.

We consider the reduced body weights of males and females to indicate a toxic effect at 2000 ppm. We assess the F_0 male low-dose weight reductions to be incidental since the weights of mid-dose males and low-dose females were comparable to controls.

We consider the significantly reduced F_0 high-dose food consumption of both males and females during the first week of the study to indicate reduced palatability of the test article rather than a toxic effect. Except for gestation and lactation values, subsequent food intake data were generally comparable for all groups and did not suggest a compound effect. We attribute the reduced food consumption of high-dose F_0 dams during both lactations and F_1 dams during the F_{2b} gestation and lactation periods to developmental effects (i.e., smaller litters, reduced pup weights), rather than parental toxicity.

Reproductive and Developmental Data: The mating data, pregnancy rates, and the numbers of females delivering and weaning their litters did not indicate any reproductive effects of the compound. Significantly increased relative testicular weights in high-dose males of both generations and mid-dose F_1 males were not associated with reduced F_0 fertility and were therefore not considered a reproductive effect.

We regard the reduced F_{1b}, F_{2a}, and F_{2b} litter sizes at birth to indicate developmental toxicity at 2000 ppm. We also consider the significantly reduced weanling weights in the high-dose group at all litter intervals and in the mid-dose group at the F_{1a}, F_{1b}, and F_{2b} intervals to be toxic effects at 650 and 2000 ppm. Although we consider the significantly increased relative kidney, liver, and testicular weights of high-dose F_{2a} and F_{2b} weanlings to be compound related, their biological meaning in terms of developmental toxicity is unclear.

B. The only major difference between the reviewers and the study authors in the interpretation of the results concerns parental relative organ weights. The study authors did not regard significant changes in relative organ weights to be compound related; however, we assess that increased relative kidney weights reflected toxicity at all dose levels and that increased relative liver weights indicated an effect at 650 and 2000 ppm.

The only other difference in interpretation concerns the food consumption data. Although the study authors noted significantly reduced food intake at 2000 ppm, we regard the differences for dams to be due to developmental effects; other differences were infrequent and did not suggest a toxic effect.

C. The study report did not include summary tables or individual data of clinical findings. This deficiency precluded the correlation of individual clinical data with other parental data and with litter data.

Item 15--see footnote *.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 21-35.

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APPENDIX A

Materials and Methods

Trifluralin toxicology reviews

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